The Journal of Laboratory and Clinical Medicine

VOL. X ST. LOUIS, MO., JANUARY, 1925 No. 4

CLINICAL AND EXPERIMENTAL

A STUDY OF THE EFFICACY OF CERTAIN ANTIDOTES IN THE TREATMENT OF ACUTE BICHLORIDE OF MERCURY POISONING*

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DURING the past ten or fifteen years a great deal of time has been spent by many investigators in an attempt to establish a reliable treatment for acute bichloride of mercury poisoning. Rabbits and dogs were the animals used in most of the experiments.

After a careful review of the literature on the subject, the following facts seem to be established: First, that clinically we have had both recovery and death from toxic doses of mercuric chloride; second, that when certain amounts of mercuric chloride are administered by mouth or subcutaneously to animals, death may or may not occur; third, that the antidotes which have been proposed by various investigators, who have obtained beneficial results with them, have been found worthless in the hands of others.

Egg albumen and milk is the most commonly used antidote in bichloride of mercury poisoning. It is often used regardless of whether the poison has been absorbed or not. We must remember, however, that albuminate of mercury is soluble in the acid of the gastric juice and when albumen is admintered, it should be removed immediately. Fantus[1] recommends a mixture of sodium hypophosphite and hydrogen peroxide; Fantus and Hyatt[2] found Carter's antidote, which is a mixture of potassium and sodium acetate, effective, if administered within five minutes after the poison had been taken; Sabbatani[3] recommends hydrogen sulphide; Hall[4] a mixture of potassium iodide, quinine and dilute hydrochloric acid; Wilms[5] reported an effective chemical antidote in calcium sulphide dissolved in sterile water and filtered, which he

*Read in abstract before the Medical Society of South Carolina, February, 26, 1924.
From the Laboratory of Pharmacology of the Medical College of the State of South Carolina, Charleston, S. C.
Received for publication April 18, 1924.

recommends to be administered intravenously, one grain of calcium sulphide given for each grain of bichloride of mercury taken. His experiments consisted in administering to dogs, one bichloride tablet and following it some time afterward by the antidote both by mouth and vein. He also reports a number of clinical observations. This antidote has been used since then with apparent success by several practitioners. Dr. E. T. Kelly, of Kingstree, South Carolina, reports a case of bichloride of mercury poisoning, in which Wilm's antidote was used with success, although the treatment was not begun until forty-eight hours after the poison was taken and four-plus albumin had appeared in the urine. Haskell and Courtney,[6] injecting subcutaneously into dogs, bichloride of mercury in fifteen mg. per kg. doses, found calcium sulphide to be of no value and gave as their opinion, that "this antidote is fraught with actual danger and it is possible that cases of bichloride poisoning may have the lethal exitus hastened rather than retarded by this drug."

Realizing the difficulty of producing a chemical detoxication in the blood stream, Burmester[7] recommended venesection and transfusion. MacNider,[8] in a series of experiments made upon dogs in which he used uranium nitrate, found "that the severity of epithelial damage to the kidney shows a correlation with the degree of acid intoxication produced by the metal, and furthermore, that the damage may, in large measure, be prevented by the intravenous injection of an alkaline solution," therefore, the use of alkalies in acute bichloride of mercury poisoning seems to be justified; Lewis and Rivers[9] used glucose and sodium bicarbonate by mouth and vein to combat acidosis. Sansum[10] found the minimum lethal intravenous dose of bichloride of mercury for dogs to be 4 mg. per kg., and, experimenting with both Carter's and Hall's antidotes, came to the conclusion that they were worthless.

Barbour[11] in a series of test tube experiments, in which he used bichloride of mercury and fresh ox serum in varying concentrations to which he added Hall's antidote, found that no precipitate occurred in concentrations as low as 1-1000. His conclusions were that this antidote was worthless because the poison has a greater affinity for the tissue than for any nontoxic remedy.

Burmester and McNally[12] administered by mouth .024 to .5 gm. of bichloride of mercury per kg. to dogs, and found that the mercury could be detected chemically in the blood of the animals in a very few minutes after its administration; that in massive intoxication, immediate renal changes, varying with the size of the dose, occurred instantly.

Haskell and coworkers[13] in a series of experiments in which they administered to dogs 10 mg. of bichloride of mercury per kg. by mouth found that the intravenous injection of twenty-five cubic centimeters of eight-tenths per cent saline solution per kg. possessed a definitely beneficial action and effects the recovery of animals by preventing absorption or hastening elimination.

Lambert and Patterson's[14] method has been used with apparent success. This form of treatment is especially used in northern hospitals. They recommend the whites of several eggs and the washing out of the stomach,

also the testing of the stomach contents, urine and feces, for mercury. As soon as the nausea is allayed the following routine:

1. The patient is given every other hour eight ounces of the following mixture: Potassium bitartrate, 1 dram; sugar, 1 dram; lactose, ½ ounce; lemon juice, 1 ounce; boiled water, 16 ounces. Eight ounces of milk are administered every alternate hour.

2. The drop method of rectal irrigation with a solution of potassium acetate, a dram to the pint is given continuously.

3. The stomach is washed out twice daily.

4. The colon is irrigated twice daily, in order to wash out whatever poison has been eliminated in that way.

5. The patient is given a daily sweat in a hot pack. They stress the importance of keeping up the treatment with the colonic drip enteroclysis day and night without interruption.

DISCUSSION OF EXPERIMENTS

This investigation was the result of several inquiries made by physicians as to the value of calcium sulphide as an antidote when administered both by mouth and intravenously.

Although both Haskell and Courtney,[6] and Wilms[5] had made reports on this antidote, the author felt justified in conducting a series of experiments in which all possible errors, in regard to the amount of absorption, would be eliminated, by injecting the poison directly into the circulation.

After placing a tourniquet on the lower part of the thigh, the poison and the antidote having been dissolved in sterile normal saline solution, were injected into a branch of the vena saphena parva on the lateral face of the leg. The injections were made through a hypodermic needle which was connected to a 50 c.c. burette by a rubber tube. In order to check up results of other investigators, the poison was also administered by mouth.

Fifty dogs and several rabbits were used in this research. The animals were divided into five groups.

Group No. 1. Was given one-half of a bichloride of mercury tablet (about 0.2 gm. or 3.5 grains) by mouth. No antidote was given in this series.

Group No. 2. Was given calcium sulphide in aqueous solution by mouth.

Group No. 3. Was treated as in group one and then given almost immediately calcium sulphide, an equal amount in aqueous solution by mouth.

Group No. 4. Was given 4 mg. of bichloride of mercury per kg.; the dose being given intravenously.

Group No. 5. Was treated as group four and then an equal amount of calcium sulphide was administered both by mouth and intravenously.

The dogs in groups one and three were given colored bichloride of mercury tablets, so that the vomitus could be watched. The disintegration of the tablet undoubtedly takes place very rapidly, because in no case was any part of the tablet vomited, although the vomitus was colored. Wilms[5] and others used one bichloride tablet by mouth; the author found that one-half of a tablet placed on the back of the tongue and washed down with water was sufficient to produce death.

Group two was given from three to seven and one-half grains of calcium sulphide to which from 50 to 100 c.c. of water had been added. The only effect noticed was that vomiting would occur unless the drug was well diluted. A solution was also made by boiling the calcium sulphide with distilled water and filtering. The filtrate was injected intravenously into both rabbits and dogs. It was found that unless a 1-1000 solution was used, and injected slowly, marked asphyxial symptoms developed; the animal dying in convulsions. On account of the insolubility of calcium sulphide, an attempt was made throughout these experiments to use sodium sulphide which is freely soluble. In the test tube both of these salts changed the mercuric chloride into the insoluble sulphide very rapidly.

The third group of dogs was given one-half of a bichloride tablet (about three and one-half grains) by mouth, followed by either sodium or calcium sulphide dissolved in 100 c.c. of water. It was found that unless this antidote is administered within three minutes after the poison it is worthless and may, if corrosion has taken place, actually hasten death. In several instances, when the antidote was administered some time after the gastro-intestinal symptoms had developed the animals died immediately; death evidently being due to shock.

It is the opinion of the author, that if administered immediately after the poison, calcium sulphide dissolved in water is of more value than egg albumen.

Knowing that colloids would delay the absorption of alkaloids, an attempt was made to determine if mucilage of acacia would not by adsorption lessen the corrosive action of the bichloride of mercury.

A series of test tube experiments were made in which bichloride of mercury was precipitated from an aqueous solution and simultaneously from an acacia solution; the percentage used being three per cent potassium iodide solution. Into test tube "A" 15 c.c. of a 1-1000 bichloride of mercury solution was placed, and six drops of 3 per cent potassium iodide were added. The usual red precipitate was the result. Then into test tube "B" 7½ c.c. of a 1-500 bichloride of mercury solution was placed. To this 7½ c.c. of 35 per cent solution of mucilage of acacia was added; thus diluting the bichloride to 1-1000. Upon addition of six drops of 3 per cent potassium iodide solution, a distinctly yellow precipitate was the result.

These experiments are of interest, and can only be explained on the theory that by adsorption the mucilage of acacia prevents the action of the potassium iodide on the mercury. This indicates that similar results should be obtained in the stomach and intestines; that is, because of the mucilage the mercury would be prevented from attacking the mucosa of the stomach and intestine. Several preliminary experiments were made to determine whether or not this theory was true.

Three and one-half grains of bichloride of mercury dissolved in 200 c.c. of U.S.P. mucilage of acacia was administered by stomach tube. The only signs of the corrosive action of the mercury was the vomiting that occurred about five minutes after the administration. The vomitus was tested for mercury, the test proving negative. One hundred c.c. of mucilage

of acacia and 30 c.c. of 25 per cent magnesium sulphate was given four hours after the poison. The animals survived.

Several animals were also given intravenously, a total of 65 mg. of mercuric chloride, followed by an intravenous injection of 5 per cent acacia in nine-tenths per cent salt solution. It was thought that since the corrosive effect is lessened in the gastrointestinal canal, when administered by mouth it would also lessen the corrosive effect upon the stomach, intestine and kidney, during the process of elimination. The animals died within twenty-four hours without showing any gastrointestinal symptoms, although when posted the mucous membrane of the stomach and intestines were found covered with mucus and the mucosa easily detached. It is very evident, from these preliminary tests, that mucilage of acacia is of value, when given by mouth, to prevent absorption and to allay inflammatory conditions. By vein it is contraindicated after absorption of the mercury.

Since Sansum[10] had established a minimum lethal intravenous dose of 4 mg. per kg. for the dog, this dose was injected into a branch of the saphenous vein of the dogs of group four, and the animals closely watched. It was observed that this amount did not always produce death, although the gastrointestinal symptoms were always present. An attempt was made then, to determine if the weight of the dog was not a factor. Several puppies and small dogs were injected in this manner, and it was found, that unless the total amount was forty or more mg., it did not produce death.

Group five was treated as in group four and followed, at times, immediately afterwards by an equal amount of either sodium or calcium sulphide, dissolved in sterile water. The antidote was ineffective. A solution of iodide of soda was also injected without effect. It is very evident to the author, from a close observation of these experiments, that when a sufficient quantity of bichloride of mercury is absorbed into the blood stream, that we have no means of combating the corrosive effect upon the mucosa of the intestine or the epithelial cells of the kidney. The animals posted in groups four and five showed marked corrosion of the walls of the small intestine, which is a sufficient cause for death, regardless of the effect upon the kidneys.

It is the opinion of many clinicians that when a toxic dose of bichloride of mercury is taken, unless treatment is administered almost immediately, death will take place eventually. That is, if the poison has been absorbed, it will produce nephritis and death. MacNider and others have made extensive studies of the effects of bichloride of mercury on the cells of the kidney and liver and undoubtedly this effect is quite a factor in producing death, but from the observations of the author in this series of experiments, it appears that the corrosive effect on the mucosa of the stomach and intestines both before and after absorption is the most important factor in the cause of death. My opinion is based on the postmortems which show conclusively that bichloride of mercury produces marked pathogenic lesions in the small intestine. Mercury seems to have a peculiar affinity for the mucous membrane of the stomach and upper intestine. This was demonstrated also by the intravenous injection of toxic doses. After administering the drug in this manner the animals developed gastrointestinal symptoms; i.e.,

bloody stools and vomitus and, when posted, evidence of the corrosive action of the drug, as it was eliminated into the intestine, was found.

The report by Lambert and Patterson,[14] of a case in which one and one-half gr. of bichloride of mercury was injected intravenously into a patient by mistake, is interesting in that it coincides with the observations made upon the animals of group four. "Eight hours after the injection of the drug, the patient vomited and the pulse became irregular and weak. The bowels began to move involuntarily and continuously and were bloody and full of mucus. Later there was almost total suppression of urine and the patient died in extreme exhaustion, five and one-half days after the injection." The essentials of the postmortem were as follows: "The kidneys were very large and deeply jaundiced. The capsule stripped with some difficulty, leaving the surface roughish. The mucous membrane of the stomach was very much swollen, very red and covered with a layer of thick mucus. The intestines were filled with a blackish watery feces, mixed with a large amount of mucus. The descending portion of the colon and sigmoid were covered with membrane and deeply congested. The lesions were most severe in the sigmoid. The anatomic diagnosis was fixed as: Hemorrhage into the lesser peritoneal sac and gastric ligaments, chronic diffuse nephritis with acute degeneration." I believe that if as much as one-fifth of a bichloride of mercury tablet (1.4 gr. or 90 mg.) is absorbed, death is inevitable.

From the experiments conducted in this research it appeared that unless corrosion took place the animal did not succumb, although quantities of albumin could be found in the urine. It was observed also that after the animal showed signs of great depression, the mere attempt to insert a stomach tube would result in death. And when an antidote which was slightly alkaline was administered in this manner, the animal would immediately die. Knowing then, the corrosive effects of the drug, both before and after absorption, a great many experiments have been made in an attempt to find some agent which would lessen this effect by changing the mercury into a less irritant form.

I believe that the recoveries that have taken place, when this or any other antidote has been used, would have occurred as well without it. It was noticed that when nontoxic doses were administered albumin regularly appeared in the urine but disappeared after a time without treatment. This acute nephritic effect is one that can also be produced by uranium and other irritant drugs.

CONCLUSIONS

1. That the minimum lethal oral or intravenous dose of bichloride of mercury for the dog cannot be based upon the weight of the animal.

2. That the lethal oral dose of bichloride of mercury for the dog is 200 mg. (about three gr.).

3. The minimum lethal intravenous dose of bichloride of mercury for the dog is 40 mg.

4. That calcium and sodium sulphides, dissolved in water and administered by mouth grain for grain, are effective antidotes for acute bichloride

of mercury poisoning, if administered before gastrointestinal symptoms have occurred.

5. That when a total dose of 40 mg. of bichloride of mercury is injected intravenously, calcium sulphide, sodium sulphide or iodide of soda is of no value as an antidote administered either orally or intravenously.

6. Mucilage of acacia will prevent the corrosive action of bichloride of mercury and delay the absorption.

TREATMENT RECOMMENDED

The author recommends the following treatment in bichloride of mercury poisoning; the procedure depending upon the amount of corrosion produced by the drug.

If the patient is seen immediately after taking the bichloride of mercury, undoubtedly the washing out of the stomach with a 1-1000 unfiltered solution of calcium sulphide is the best treatment. This should be followed by the administration of about 300 c.c. of mucilage of acacia U.S.P. If vomiting has already occurred, which is the first effect of the corrosive action of the drug, the same treatment would be used, but if other gastrointestinal symptoms are present, calcium sulphide is contraindicated. The stomach should be washed out with care and quantities of mucilage of acacia given both by mouth and by high colonic injection. A mild cathartic should also be given. If the patient develops anuria, efforts should be made to restore this function. A mixture of acetate of potash, caffein citrate and infusion of digitalis should be given every four hours for several days.

Other well-known methods of elimination should be used. Mercury is absorbed as soluble organic compounds of proteins and is deposited in various organs of the body. Sollman[15] says, after long continued administration, the excretion persists intermittently for many months. It is excreted mainly in the urine and feces. It is, undoubtedly, also secreted by the stomach. Lambert and Patterson[14] recommend testing, at regular intervals, the stomach contents, urine and feces, for mercury. As the whole treatment depends upon the absorption and elimination, this is very necessary. As long as the mercury is found in the secretions, the diuretic mixture should be continued. Three hundred c.c. of mucilage of acacia should also be given at least once a day by mouth and 100 c.c. by high colonic injection. The mucilage will prevent the reabsorption of the mercury from the stomach and intestines. There are several tests for mercury. Klein's is considered very delicate. Add a little KI, a drop of ammonium chloride and then NaOH drop by drop: brown or yellow color or precipitate (NHg_2I).

Finally, the author does not believe that a satisfactory antidote has been found which when injected intravenously will change the mercury into a less corrosive salt. The whole treatment of acute mercury poisoning should be aimed against absorption, to prevent the local effect, and to increase as rapidly as possible the elimination and prevent the reabsorption of the drug.

The author wishes to express his appreciation of the valuable assistance of the following members of the faculty: Dr. Francis B. Johnson of the

Department of Clinical Pathology, Dr. Henry Plowden of the Department of Pathology, Dr. H. Grady Callison and Mr. E. C. Hood of the Department of Pharmacology.

In the following, additional detail is given in regard to some of the experiments.

GROUP 1.

Dog No. 52. Weight 5 Kg.

First day,	11.57 A. M.	One-half bichloride of mercury tablet (about three and one-half grains) followed by 100 c.c. of water, given by mouth.
	12.02 P. M.	Dog vomits, vomitus shows bluish color due to color of tablet.
	3.30 P. M.	Dog living but appears to be in shock.
Second day,		Dog died during the night.

GROUP 4.

Dog. No. 17. Weight 10.6 Kg.

First day,	5. P. M.	Drug: Bichloride of mercury, 4 mg. per kg. intravenously. The poison was dissolved in .9 per cent salt solution and injected into a branch of the saphenous vein. Total dose 42.4 mg.
	5.10 P. M.	Dog vomits.
Second day,	10. A. M.	Bloody feces noted in cage.
	4. P. M.	Passes urine.
Third day,	10. A. M.	Specimen of urine collected and examined. Contaminated with feces, containing a quantity of blood.
Fourth day,	10. A. M.	No improvement.
Fifth day,		Dog died during night.

GROUP 5.

Dog. No. 21. Weight 7.5 Kg.

First day,	5.40 P. M.	Drug: Bichloride of mercury, 4 mg. per kg. intravenously. The poison was dissolved in .9 per cent salt solution and injected into a branch of the saphenous vein.
Second day,	10.30 A. M.	Profuse defecation, showing blood. Evidences of vomiting during night.
	1. P. M.	Calcium sulphide, 30 mg. dissolved in distilled water after boiling for 15 minutes, filtered, and injected into a branch of saphenous vein.
	3.45 P. M.	65 mg. calcium sulphide orally, vomiting occurring immediately.
	5. P. M.	Dog weak, respiration shallow, heart rate rapid.
Third day,		Dog died during night.
		Post: Liver apparently normal, intestines markedly corroded. Mucosa strips easily from serous coat. Hemorrhagic spots with apparent perforations present in walls of small intestine. Kidneys swollen, dark purplish in color, showing areas of hemorrhage.

SPECIAL EXPERIMENT.

Dog No. 29, (Small Puppy). Weight 1.5 Kg.

First day,	11.50 P. M.	Drug: Bichloride of mercury, 4 mg. per kg. intravenously. The poison was dissolved in .9 per cent salt solution and injected into a branch of the saphenous vein. Total dose: 6 mg.
	12.40 P. M.	Small greenish stool noted.
Second day,		Animal fussy.
Third day,		No change.
Fourth day,		Animal lively. Site of injection sloughed slightly. Cleaned and dressed with ichthyol ointment.
Fifth day,		Animal seems weak.
Sixth day,		No change.

Seventh day,	Animal vomiting at intervals, passing worms.
Eighth day,	Improved.
One month after,	Alive, legs much improved.

NOTE: Since the above article was received for publication I concurred with Dr. Leland B. Salters, of the Florence Infirmary, who tried the treatment recommended, with success. He writes, "I want to thank you for the information given in this case because without it I do not know just what course I would have taken."

The patient, aged twenty-three, white, male, single, following a period of whiskey drinking, swallowed three seven-and-one-half gr. bichloride of mercury tablets on Monday, June 9, 1924, at eleven A. M. Treatment was commenced twenty-five minutes after swallowing the tablets.

An hour after taking the mercury, his condition was as follows: A tall, muscular youth, weighing about 130 pounds, and apparently without anatomical defect, prostrated to the degree at which he could barely stand. Drops of cold perspiration running off forehead and face, and all clothing thoroughly soaked with perspiration. Skin and mucous membrane pale, features pinched, face has a slight cyanotic tinge. No corrosion about the mouth; mentally clear and manageable, vomiting every few minutes,—vomitus consisting of bloody water at first, later bile was present. Eyes deep set and encircled with dark rings; complains constantly of cramps in thighs and calves; has no abdominal pain. Slight pain at intervals in precordium; mouth temperature 97, respiration 24, pulse 90, very compressible and uniform in force, frequency and volume. Heart sounds distinct, no murmur or irregularity. Systolic pressure 110, diastolic 85. Abdomen flat, soft and painless. All extremities move freely; no cutaneous eruption anywhere. Very restless and exceedingly thirsty, though most of the water he drank would be immediately vomited. Urinalysis at this time showed albumin two-plus, casts three-plus, no sugar, no acetone, no pus, no crystals. Three hours later, a bloody diarrhea set in; there was no associated griping.

The patient was in the Infirmary eleven days. The cramps continued the first six days. Bloody vomit continued three days; bloody stools three and one-half days. Temperature remained above normal throughout his stay in the Infirmary,—highest reached, 101 the second day.

RECORD OF URINALYSIS

June 9: Spgr. 1009; Alb. 2-plus; casts 3-plus; acetone none; pus none; crystals 2-plus.
June 10: Alb. 3-plus; casts 3-plus; acetone none; pus none; crystals 2-plus.
June 11: Spgr. 1028; Alb. 4-plus; casts 3-plus; acetone trace; pus 1-plus; crystals 3-plus.
June 12: Spgr. 1019; Alb. 3-plus; casts 2-plus; acetone trace; pus 2-plus; crystals 4-plus.
June 13: Spgr. 1015; Alb. 3-plus; casts 2-plus; acetone none; pus 2-plus; crystals 4-plus.
June 14: Spgr. 1008; Alb. 2-plus; casts 2-plus; acetone 2-plus; pus 3-plus; crystals 4-plus.
June 15: Spgr. 1007; Alb. 1 plus; casts none; acetone 3-plus; pus 2-plus; crystals 4-plus.
June 16: Spgr. 1005; Alb. trace; casts none; acetone 3-plus; pus 1-plus; crystals 2-plus.
June 17: Spgr. 1011; Alb. none; casts none; acetone 2-plus; pus none; crystals 4-plus.
June 18: Spgr. 1015; Alb. none; casts none; acetone 2-plus; pus none; crystals 4-plus.
June 19: Spgr. 1018; Alb. none; casts none; acetone 1-plus; pus none; crystals 4-plus.
July 18: Spgr. 1018; Alb. none; casts none; acetone none; pus none; crystals none.
July 21: Spgr. 1020; Alb. none; casts none; acetone none; pus none; crystals none.

Only granular casts were found, no blood casts were ever found. Traces of blood by chemical test were discovered the second and third days.

Urinary Output.—Nine, three ounces; 10, fifteen ounces; 11, twenty-two ounces; 12, thirty-three ounces; 13, forty-three ounces; 14, sixty-nine ounces; 15, fifty ounces; 16, thirty-four ounces; 17, fifty-eight ounces; 18, forty-four ounces; 19, sixty ounces.

Treatment.—After the primary stomach washing, the stomach tube was laid aside and used no more. Throughout the attack he was advised to drink all the water possible,—the quantity retained varying from day to day. Every day he received an ounce of mineral oil, morning and night. He was given mucilage of acacia, three ounces, every four hours for five days, then three times daily for six days. Potassium acetate thirty grains in eight ounces

of water was given by enteroclysis every four hours for six days, after which renal function was encouraged by fifteen grains each of potassium acetate and citrate in a glass of water by mouth every four hours. An ampule of digifoline was given hypodermically every four hours for four days. On the 10, 11, and 12 Fischer's solution was given intravenously in the following quantities: 850 c.c.; 900 c.c.; 700 c.c. From the fourth day on Dobell's solution was used as a mouth wash. He took no food the first five days. Milk was given for the next five days, when soft egg and cereals were added. As you will see from the urinalysis, his kindneys cleared up rather promptly and examination today showed a perfectly normal urine.

July 21. The patient is back at work, looking well and feeling fine. He eats anything he wants and has no gastrointestinal disturbances resulting.

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Rockefeller Institute of Medical Research

The Rockefeller Institute for Medical Research has announced the release of the drug known as Tryparsamide for use in the treatment of human and animal trypanosomiasis (African sleeping sickness and *mal de caderas*) and selected cases of syphilis of the central nervous system. This action is based on results reported from clinical investigations which have been in progress for several years. The drug will be manufactured by the Powers-Weightman-Rosengarten Co. of Philadelphia, and will become available through the regular trade channels about January 1, 1925. In releasing the drug for the benefit of the public, the Rockefeller Institute desires it to be known that the Institute does not share in any way in profits that may be derived from the sale of the drug and that, with the cordial cooperation of the manufacturers, provision has been made for the maintenance of a schedule of prices on as low a basis as possible.

THE EFFECT OF HEMOLYSIS ON THE CALCIUM AND INORGANIC PHOSPHORUS CONTENT OF SERUM AND PLASMA*

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IN articles dealing with the calcium and phosphorus content of blood it is often very difficult to ascertain whether the author used serum or plasma as a basis for his conclusions. Often, indeed, the two are used synonymously. In an article published in 1922, for instance, the heading of a table is given as follows: "Concentration of Na (or K, Ca and Mg) in Blood Serum and Corpuscles of Normal Adults," but the heading in the table reads "Plasma" instead of serum.[1] Many similar instances might be cited. We therefore undertook to determine whether the calcium and inorganic phosphorus content of serum and plasma were identical or varied, and what the trend of variation might be. Since blood for quantitative chemical estimations may show varying degrees of hemolysis, it is important to know also whether this factor has any bearing upon quantitative results. Further comparative determinations were, therefore, made on serum and plasma from hemolyzed and nonhemolyzed blood.

Experimental.—Two samples of blood of approximately 30 c.c. each were drawn from a sheep. One was prevented from clotting by the addition of sodium citrate in the proportion of 40 mg. to 10 c.c. of blood. Each sample was subdivided into two portions and one portion of each was hemolyzed by the addition of three drops of water. Calcium and inorganic phosphorus were determined on the centrifuged supernatant. For the determination of calcium, Kramer and Tisdall's[2] method was followed. The method of the same authors[3] was used for calcium in hemolyzed blood. For inorganic phosphorus Briggs'[4] modification of the Bell-Doisy phosphate method was employed. Determinations were made immediately after the blood was drawn, and the figures reported are the averages of duplicates. Two sheep furnished the blood used.

Discussion.—The table shows that there is no difference in the amount of inorganic phosphorus present in serum and in plasma. This is in agreement with the work reported by Tolstoi.[5] Hemolysis does not affect the phosphorus content of either the serum or the plasma. The calcium content, however, showed consistently somewhat lower figures for plasma than for serum. This was true in nineteen of twenty-three specimens examined, which gave values of 0.2 mg. to 3.4 mg. lower than the corresponding tests on serum. The four remaining cases showed a difference in serum and plasma calcium which was within experimental error. Hemolyzed blood invariably gave a

*From the Gusta Morris Rothschild Fund of the Michael Reese Hospital and the Nelson Morris Memorial Institute for Medical Research, Chicago.

Received for publication, July 12, 1924.

TABLE I

CALCIUM AND PHOSPHORUS CONTENT OF HEMOLYZED AND NONHEMOLYZED SERUM AND PLASMA*

	CALCIUM						PHOSPHORUS (INORGANIC)			
	NONHEMOLYZED		HEMOLYZED				NONHEMOLYZED		HEMOLYZED	
			UNTREATED		TREATED WITH AMMONIUM ACETATE					
NUMBER	SERUM	PLASMA	SERUM	PLASMA	SERUM	PLASMA	SERUM	PLASMA	SERUM	PLASMA
1	11.5	8.2		10.5	9.2					
2	11.0			9.9	8.2	9.7	6.2		6.1	5.9
3	12.3	11.7	15.2	10.9	13.1	10.6	6.3	6.5	6.4	6.7
4	11.6	12.3	13.7	11.4	9.7	9.9	4.3	3.9	4.0	4.0
5	12.8	11.2	17.6	11.0	12.5					
6	15.1	11.9	18.2	15.2	14.3	9.9	5.8	5.5	5.6	5.2
7	13.8	12.6	16.7	13.9	10.4	10.4	5.9	5.9	5.9	5.2
8	15.3	13.3	17.5	14.8	14.9	11.1	6.0	6.0	6.0	5.9
9	11.6	11.3	17.7	16.7	13.7	12.3	4.4	4.5	4.7	4.8
10	12.5	11.6	16.6	16.3	11.6		5.5	5.6	5.7	5.2
11	16.2	12.8	18.3	15.3	14.1	11.0	3.8	3.7	3.7	3.7
12	14.0	13.3			13.1	10.4	4.8	4.4	4.6	4.4
13	15.3	13.9			13.8	12.5	4.5		4.6	4.3
14	16.5	16.3			14.0	13.9	5.0		5.1	4.6
15	13.2	13.5			11.1	11.0	4.8		4.7	4.3
16	13.6	12.8			12.8	10.6	4.6		4.9	4.8
17	12.8	12.1			12.8	12.0	4.7		4.7	4.8
18	12.2	11.9			11.1	10.8	4.8		4.8	4.8
19	11.3	10.1			11.2	11.0	5.0		4.9	5.0
20	13.4	13.1			12.4	11.1	4.8		4.7	4.7
21	12.4	11.5			11.5	12.1	4.7		4.9	4.8
22	14.2	13.0			12.8	11.6	5.0		4.8	5.0
23	12.8	13.0	—		11.8	10.9	4.7		4.8	4.8
24	12.7	13.0			11.1	11.6	4.9		4.9	4.9

*Reported as mg. per 100 c.c. of blood.

higher calcium content in serum than nonhemolyzed. Slight hemolysis does not necessarily affect the amount of plasma calcium; thus, we found that out of ten specimens, three gave a slightly lower figure and seven higher than the nonhemolyzed blood. The percentage increase of calcium in hemolyzed serum was from 14 per cent to 53 per cent over that of nonhemolyzed serum; the corresponding increases for plasma were 10 per cent to 48 per cent over the nonhemolyzed plasma. Strange to say, in only two cases did the calcium content of hemolyzed plasma exceed that of nonhemolyzed serum.

Kramer and Tisdall[3] stated that hemolyzed serum might be used for calcium determinations if treated with ammonium acetate. We accordingly treated both the hemolyzed serum and hemolyzed plasma by their method and determined the calcium content. Our results show lower calcium values in both serum and plasma when hemolyzed and treated with ammonium acetate than in the nonhemolyzed serum and plasma. Twenty-one out of twenty-four serum specimens showed values ranging from 0.9 per cent to 25 per cent lower than the nonhemolyzed serum. Of twenty specimens of plasma, seventeen gave values ranging from 0.8 per cent to 22 per cent lower than the nonhemolyzed plasma. The percentage variation is summarized in Table II. The difference is very likely due to the fact that in precipitating the iron from the hemolyzed serum or plasma, some of the calcium is likewise brought down. This difficulty might be overcome by reprecipita-

TABLE II

COMPARISON OF SERUM AND PLASMA CALCIUM IN HEMOLYZED AND NONHEMOLYZED BLOOD

	INCREASE OF SERUM Ca OVER PLASMA Ca		INCREASE OF HEMOLYZED SERUM Ca OVER NONHEMOLYZED SERUM Ca		INCREASE OF HEMOLYZED PLASMA Ca OVER NONHEMOLYZED PLASMA Ca		INCREASE OF NONHEMOLYZED SERUM Ca OVER TREATED HEMOLYZED SERUM Ca		INCREASE OF NONHEMOLYZED PLASMA Ca OVER TREATED HEMOLYZED PLASMA Ca	
NO.	mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent
1	+3.3	+40.2			+2.3	+28.1	+2.3	+25.0		
2							+2.8	+34.2		
3	+0.6	+ 5.1	+2.9	+23.6	−0.8	− 6.8	−0.8	− 6.1	+1.1	+10.4
4	−0.7	− 5.7	+2.1	+18.1	−0.9	− 7.3	+1.9	+19.6	+2.4	+24.2
5	+1.6	+14.3	+4.8	+37.5	−0.2	− 1.8	+0.3	+ 2.4		
6	+3.2	+26.9	+3.1	+20.5	+3.3	+27.5	+0.8	+ 5.6	+2.0	+20.2
7	+1.2	+ 9.5	+2.9	+21.0	+1.3	+10.3	+3.4	+32.7	+2.2	+21.2
8	+2.0	+15.0	+2.2	+14.8	+1.5	+11.3	+0.4	+ 2.7	+2.2	+19.8
9	+0.3	+ 2.6	+6.1	+52.5	+5.4	+47.8	−2.1	−15.3	−1.0	− 8.1
10	+0.9	+ 7.8	+4.1	+32.8	+4.7	+40.5	+0.9	+ 7.8		
11	+3.4	+26.4	+2.1	+13.0	+2.5	+19.5	+2.1	+14.9	+1.8	+16.3
12	+0.7	+ 5.3					+0.9	+ 6.9	+2.9	+27.9
13	+1.4	+10.1					+1.5	+10.9	+1.4	+11.2
14	+0.2	+ 1.2					+2.5	+17.7	+2.4	+17.3
15	−0.2	− 1.2					+2.1	+18.8	+2.5	+22.7
16	+0.8	+ 5.9					+0.8	+ 6.3	+2.4	+20.8
17	+0.7	+ 5.8					0	0	+0.1	+ 0.8
18	+0.3	+ 2.5					+1.1	+ 9.9	+1.1	+10.2
19	+1.2	+11.9					+0.1	+ 0.9	−0.9	− 8.2
20	+0.3	+ 2.3					+1.0	+ 8.1	+2.0	+16.5
21	+0.9	+ 7.8					+0.9	+ 7.8	−0.6	− 5.0
22	+1.2	+ 9.3					+1.4	+10.9	+1.4	+12.1
23	−0.2	− 1.5					+1.0	+ 8.5	+2.1	+19.3
24	−0.3	− 2.3					+1.6	+14.4	+1.4	+12.1

tion, but it would involve much more manipulation and would increase the possibility of error.

Our findings show that it is not advisable to confuse the terms serum and plasma or to use them interchangeably when referring to calcium content, since there is some variation in the two cases. It is also well to keep clear the distinction between the hemolyzed and nonhemolyzed serum and plasma, as there is always a difference here in the calcium content. We would recommend that in reporting experiments on blood calcium, only nonhemolyzed blood be used, and serum or plasma specified.

SUMMARY

1. There is no difference in the phosphorus content of serum and plasma in either hemolyzed or nonhemolyzed blood.

2. The calcium content of nonhemolyzed serum is slightly higher than that of plasma. Hemolyzed blood, after being treated with ammonium acetate, gives a slightly lower calcium content than nonhemolyzed blood.

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SPINOUS CELL CARCINOMA*

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THE spinous cell carcinoma or, carcinoma developed from the stratum spinosum of stratified squamous epithelium has only recently been recognized as a distinct entity. Its position in the galaxy of tumors has not yet been generally accepted. This paper is presented to call your attention to some of the facts regarding this very common neoplasm, not only for what might be termed as an academic interest of exact identification and classification, but also for the practical reason that it is being recognized that tumors have definite habits and that it is as important to know the kind and origin of a tumor as it is to know that a given lesion is a tumor.

Ewing in his treatment of "Neoplastic Diseases" does not mention the spinous cell carcinoma as such, but includes this type of tumor in a heterogeneous group to which he gives the name of acanthoma. McCallum in his recent textbook on pathology includes it under an equally heterogeneous group of squamous cell carcinoma. The reason perhaps that this tumor has not been recognized, is, in the first place a general satisfaction on the part of clinicians and pathologists with the fact that a certain lesion is a carcinoma without any particular attention as to what class of tissues composes the tumor; and in the second place somewhat of a misapprehension has been perpetuated by histologists as to the manner of development and production of the various layers of stratified squamous epithelium, so that it has not occurred to the pupils of these histologists, who later became pathologists, that there was a possibility of a tumor developing from this particular layer.

Histologists have told us and still do tell us that in stratified squamous epithelium the act of multiplication takes place in the stratum germinativum, and that the cells so produced must of necessity be crowded outwards, and, in the process are modified, forming the stratum spinosum and finally become desiccated and keratinized to form the stratum corneum. Some few histologists however, have admitted that reproduction does take place in the very lowest layer of the stratum spinosum. A careful study of this type of epithelium reveals the fact that active reproductive processes are found as

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*Read before The Third Annual Meeting of the American Society of Clinical Pathologists, Rochester, Minn., June 5-7, 1924.

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far as the middle of this layer if not farther, and if we would add the facts of tumor formation as any indication of histologic processes, since in the so-called basal cell carcinoma we find little evidence of production of other types of cells of stratified squamous epithelium than those of the basal layer, we would be forced to conclude that the larger part of the stratum spinosum is produced by reproduction of the cells of this layer.

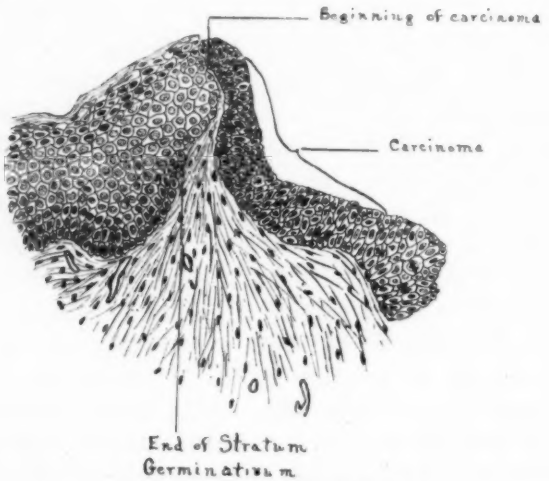
If we make a careful study of the factors which enter into the inciting of tissues to neoplastic growth and study the probability of the occurrence of the application of these facts to the various tissues, we would be led to the conclusion *a priori* that the stratum spinosum is the one from which we would expect the majority of tumors arising from stratified squamous epithelium to originate. Of such factors we would expect, first, to find tumors in tissues which normally preserved and exercised a rather high rate of reproductive processes, which is one of the reasons why epithelial and connective tissue tumors are the most common. Another fact would be that tumors arise from those tissues which are least stable, or in other words, have a rather weak or easily disturbed tissue set, which again would include the epithelium and connective tissues. Another fact would be the separation of living cells from an immediate supply of nourishment which would also include a supply of those internal secretions, which make for orderly development and reproduction. This fact coupled with the biologic law of an extra attempt at reproduction under the circumstances of unfavorable conditions for the carrying on of the vital functions would again affect most potently any tissue in a position similar to that of stratum spinosum. For here we have a tissue of living cells which must of necessity get their nourishment by means of percolation of nutritive fluids for some considerable distance from the blood vessels, and these same cells, being crowded farther and farther away from such meager supply, would not only suffer from reduced nutrition, but would be correspondingly freed from the controls to reproduction and its orderly progression, therefore readily grow wild or neoplastic.

If now we study the possibilities of tumor formation from stratified squamous epithelium, since there are two layers of living cells in this epithelium, we would expect to find at least three distinct types of tumors: one in which all three layers were represented in approximate equal proportions or the epidermoid carcinoma, one composed wholly or almost wholly of cells of the basal layer, or carcinoma basocellulare, and one in which the cells are all or for the greater part those arising from the stratum spinosum, or spinous cell carcinoma.

Two of these types have long been recognized. The third type has been but recently recognized by a few pathologists and the employment of this term is by no means general. Ewing's acanthoma includes the epidermoid carcinoma and the spinous cell carcinoma. The term of squamous cell carcinoma used by some pathologists is indefensible when applied to tumors arising from this epithelium for the reason that the only squamous cells in this type of epithelium are dead and hence cannot form tumors. If there is a squamous cell carcinoma at all it would be the endothelioma of Mallory, or in other

words the tumor arising from simple squamous epithelium. The mosaic appearance of the cells both in the stratum spinosum of stratified squamous epithelium and in the tumors arising from it is not confined to tumors arising from this tissue alone, but is characteristic of carcinomata arising from any epithelium. Likewise, the employment of the term, "squamous" applied to this form of epithelium by the histologists can only be defended on the grounds of failing to recognize the third dimension in their concept of tissues, forgetting that they are dealing with irregular polyhedrons rather than polygons. Such a mosaic picture can be obtained from a transverse section of cylindrical or cuboidal epithelium.

The spinous cell carcinoma is composed of fusiform polyhedral cells which in typical cases are approximately twice as long as broad. They have granular cytoplasm, and oval vesicular nuclei of comparatively good size. Although the cytoplasm is basophilic, or neutrophilic the nucleus is easily



Drawing to explain Fig. 1.

made out. These cells tend to form sheets composed of many layers of cells. The longer axis of the cells of the middle layers of the sheets tends to be at right angles to the direction of growth. The longer axis of the border cells tends often to be parallel with the direction of growth. Sometimes, however, the sheet tendency is not apparent, the cells being arranged in great masses. and because of the destruction of all tissue up to and even including the walls of the blood and lymph vessels, these tumors are frequently diagnosed as sarcomata.

The typical features of this neoplasm, of course, are modified by the opportunity of the the tumor tissue to free and unhampered growth. If the tumor is invading a very dense tissue such as the corpus cavernosum the cells are often very small, densely packed together, with apparently very little or no cytoplasm, and might be easily mistaken for a small, dense, spindle cell sarcoma. We sometimes find in these carcinomata a tendency to pearl formation, but this is absent in rapidly growing tumors. Rarely are there any cells resembling those of the stratum germinativum.

There is often a more or less dense infiltration of lymphocytes, endothelial cells, connective tissue eosinophiles, and even polymorphonuclear leucocytes not only in the neighborhood, but within the tumor cell masses, which often confuse the picture. These tumors do not necrose as early or to such an extent as would other carcinomata in which the cell masses are as large. This is probably due to the fact that the spinous cell is one that is used to scant nutrition.

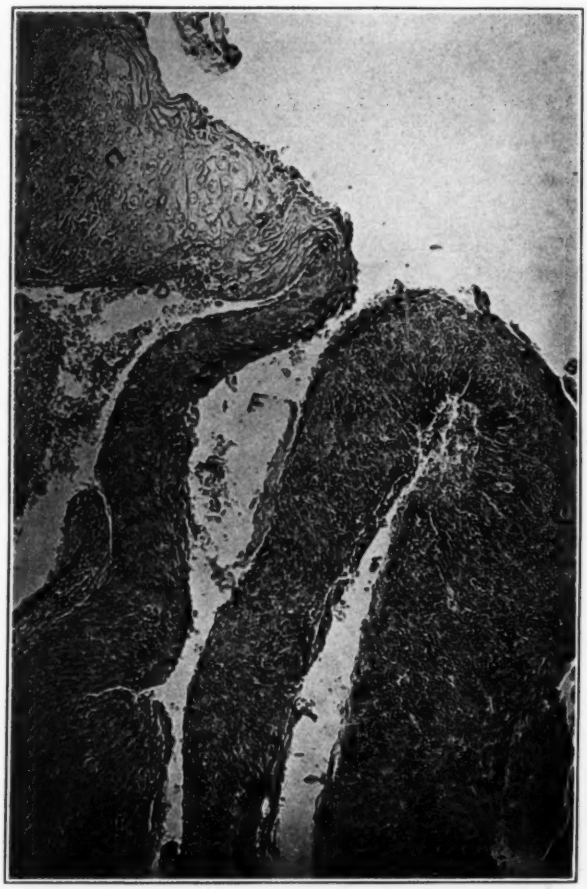


Fig. 1.—Showing origin of spinous cell carcinoma, (cervix uteri) x150. *A-B*, Normal surface of organ; *C*, hyperplastic epithelium; *D*, end of stratum germinativum; *E*, beginning of neoplasia; *F*, space left by necrosis.

The spinous cell carcinoma is rather slow in growth compared to cylindrical cell carcinoma. It destroys all tissues it penetrates and invades all tissues, yet it does not metastasize as readily as do most carcinomata. It has been known to destroy the cervix, upper vagina, part of the corpus uteri, and broad ligament, and invade the bladder and rectum without demonstrable metastasis. This late metastasis is probably due to the manner of growth which may be likened more to a liquid flowing over a surface rather than to a worm crawling along a burrow. That is, the spinous cell carcinoma crowds its way as a mass between the other tissues instead of penetrating

the tissues as slender cords of cells as most other carcinomata do, so that the detachment of individual cells or groups of cells from the masses is difficult, whereas the detachment of individual cells or groups of cells from slender cords is very easy.

These tumors sometimes appear as growths of short papilla above the surface, or as excavations in which the walls and floor are composed of stalactites. This appearance is due more to the necrosis of the invaded tissue between the convoluted sheets of tumor tissue than to a real papilla forma-

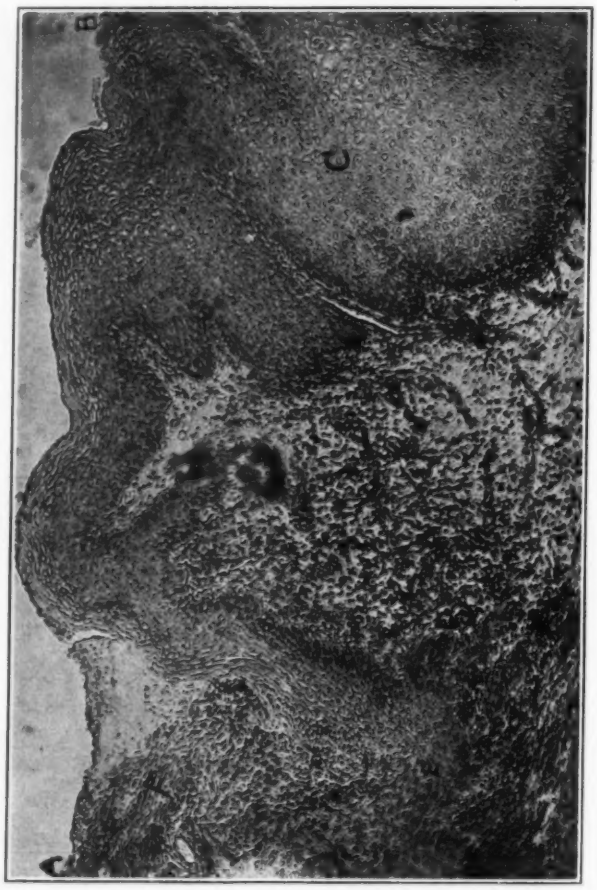


Fig. 2.—Showing origin of spinous cell carcinoma, burrowing type, x150. *A-B*, Surface of organ; *C*, hyperplastic epithelium; *D*, end of stratum germinativum; *E*, beginning of neoplasia; *F*, atrophied epithelium.

tion; however the true papilla formation is sometimes present. But occasionally, instead of appearing on the surface the tumor burrows deep into the tissues causing widespread destruction of other structures and leaving no trace or sign on the surface of its existence. It not infrequently happens that surgeons and gynecologists overlook this burrowing type, not suspecting the presence of the tumor because of the lack of disturbance of the surface. In my own experience as pathologist I have seen a number of such cases in which the cervix was amputated for other reasons, the presence of the carcinoma being revealed only on tissue examination.

The spinous cell carcinoma can and does occur on any part of the body where stratified squamous epithelium is found. It is most common, however, in those localities where the epithelium is kept moist, in other words on the interior, such as the mouth, esophagus, anus, vagina, and cervix uteri. In the latter site it is the most common tumor. In this location also it is found in its purest form.

The etiologic factors which seem most potent in the origin of these tumors are long-standing mild inflammation or trauma, or both. In the cervix it

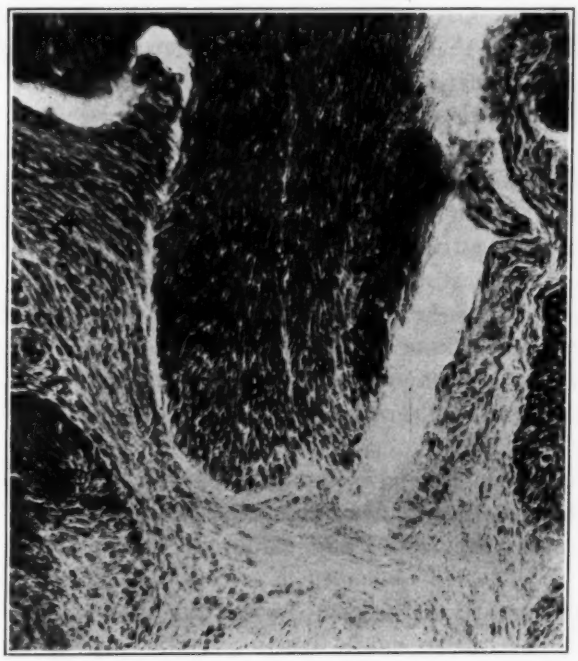


Fig. 3.—Spinous cell carcinoma invading corpus cavernosum, x300. *A*, Connective tissue of organ; *B*, carcinoma.

is chronic cervicitis or endocervicitis alone, or plus erosion, or the previously mentioned inflammatory conditions involving an area of cervical repair where the epithelium has been turned in.

It is the province of this paper to deal almost wholly with the spinous cell carcinoma in its typical form. There are, of course, many modifications and combinations of this with other forms met, as is true of all tumors. But if those of you who do tissue work will bear in mind the points brought out, I am sure you will be surprised, if you have not already been so, at the frequency of the occurrence of this type of tumor, and also I am sure you will be convinced of its histogenesis. My own attention was first called to it in an accidental case where the origin of the tumor was very apparent.

(*For discussion, see p.* 325.)

AN ADEQUATE LABORATORY SERVICE FOR THE HOSPITAL*

By Philip Hillkowitz, B.S., M.D., Denver, Colo.

ONE of the most important requisites for a well-conducted institution for the care of the sick is the provision of proper laboratory facilities. From the very beginning of the movement for standardization of hospitals it was recognized that no exact scientific work in the diagnosis and treatment of disease could be carried out without the aid of the laboratory.

Paragraph 4 of the list of minimum standards of requirements enunciated by the American College of Surgeons reads: "That clinical laboratory facilities be available for the study, diagnosis and treatment of patients, these facilities to include at least chemical, bacteriologic, serologic, histologic, radiographic and fluoroscopic service in charge of trained technicians."

The College has wisely refrained from laying down definite or detailed specifications as to the kind or amount of clinical laboratory service to be given in the various classes of cases that are admitted to the hospital. It has left it to time and to the discretion of each hospital to work out its own problem in this particular.

A sufficient period has now elapsed since the inauguration of the standardization movement to advance a tentative program as to what constitutes adequate laboratory service. Daily conference with clinicians and surgeons and exchange of views between them and the pathologist during the past three years have greatly helped to clarify the question and to formulate certain basic principles which should invite universal approval.

We may first consider the so-called routine laboratory examinations, performed on every patient admitted to the hospital, regardless of the nature of the ailment. A clinical and microscopic examination of the urine comprising the determination of the specific gravity, reaction, the test for albumin and sugar and a search for casts, pus and crystals, is of course a *sine qua non* in every modern hospital. No patient, except in emergency cases, should go to the operating table or under the anesthetic without the accompanying report of the results of the urinalysis. It is as much part and parcel of hospital routine as obtaining the temperature and pulse, a procedure to which the patient is entitled by right as included in his implied contract with the hospital management. Next comes an examination of the blood. The extent of the investigation naturally varies with the case, and there is divergent opinion as to what determinations should be carried out routinely. On the one hand we must, in the interest of efficiency, avoid unnecessary and useless examinations which are time-consuming and constitute a great strain on the working personnel in the laboratory besides taking their energy away from

*Read at the Meeting of the Western Section of the American College of Surgeons, Denver, Colorado, October 5, 1923.
Received for publication, June 20, 1924.

more essential duties. On the other hand, however, we cannot, in our zeal for economy of effort, overlook the possible accidental discovery of unlooked for important findings in more elaborate routine examinations. Between the Scylla and Charybdis we have to steer a middle course. It is, therefore, conceded that a white count of the blood is indicated in every case, this being substituted by a determination of the coagulation time in the ordinary tonsillectomy cases. Where the enumeration of the white blood corpuscles exceeds the normal a differential count should be made as a routine measure.

Another basic principle, of greatest importance to the surgeon, is the obligatory examination by a pathologist of all tissues removed at operations.

The examinations above specified of the urine, blood and tissues constitute what may be termed the basic routine laboratory examinations. These alone, however, would not fulfill in letter or spirit the requirements of adequate laboratory service. The enormous strides made in the diagnosis of disease by calling into requisition the recent contributions of chemistry, bacteriology, immunology and allied sciences make the laboratory an indispensable component of the hospital's armamentarium, to be placed at the disposal of the profession. Hence the equipment and personnel should be such as to enable the performance of the various laboratory tests employed in the modern practice of medicine when these are required by the attending physician. Accurate quantitative determination of urinary constituents, the examination of gastric contents and of feces and cerebrospinal fluid; the newer methods of blood chemistry, serologic procedures such as the Widal reaction and Wassermann test, blood cultures, preparation of vaccines and similar bacteriologic and clinical microscopic procedures ought to be carried out in the modern hospital laboratory.

Some of the more complicated tests, performed at infrequent intervals, requiring special apparatus, need not necessarily be done on the hospital premises, but may be performed by the pathologist in his private laboratory if his own facilities be better.

The examination of tissue removed at operation or for diagnosis is one of the most important functions of the hospital pathologist. There is no need to emphasize the value to the surgeon of the microscopic examination of histologic sections. Occasionally, a voice is heard in deprecation of the necessity of sectioning a manifestly gangrenous appendix and still louder are the protests against sectioning tonsils. The objections, be it mentioned parenthetically, are not against the scientific procedure *per se,* but against the cost of the examination added on the patient's already burdensome hospital bill. Of this, however, more anon. Leaving tonsils for the present out of consideration as a mooted question, we may lay it down as axiomatic that all tissues should be examined both macroscopically and microscopically. With the best of scrutiny and expert naked eye review of a specimen, some conditions will escape observation that would be revealed by the microscope. If tissues were examined only when requested by the surgeon, we should then be back in the pre-standardization era. Letting down the bars on certain kinds of tissue would quickly land us there. For this reason, we would make no exception in the case of tonsils

which are worthy of the same respect as the appendix or the ovary. And while utilitarian value to patient may not be immediately apparent in every case, both the physician and the patient are happier in the consciousness that all the scientific armor of the laboratory has been brought to bear on the case.

We are wont to speak of the laboratory somewhat metaphorically as an impersonal thing. Just as in referring to the operating room we desire principally to imply the surgeon, so in our use of the word "laboratory," we really designate the pathologist.

His rôle in the hospital is not merely to carry out or supervise the performance of the various routine or requested tests, but to interpret the results in terms of the patients. The clinician finds the pathologist willing to see or talk the case over with him, ready to suggest some laboratory procedure that would throw light on an obscure diagnosis. Together, by coordinating the clinical and laboratory findings, they are able to utilize their talents for the benefit of the patient. In short, the pathologist is a consultant in medicine. What is said here of the pathologist and the clinical laboratory applies with equal force to the roentgenologist and the x-ray laboratory. We, therefore, consider the wording of the minimum requirement for laboratories, "under the charge of trained technicians," rather unfortunate,* for no matter how highly trained they may be (and the training is anyhow mostly obtained from the pathologist) they have their limitations and should not be entrusted with such a grave responsibility as interpretation of laboratory determinations. To the stereotyped rejoinder that the clinician can do his own interpretation of clinical and x-ray laboratory findings, we may adduce the evidence that with the multiplicity of knowledge that has to be mastered nowadays by the physician only few can possess the happy faculty of keeping abreast of the advance in laboratory research.

It may be said in extenuation of the modest requirement of a trained technician rather than of the ideal, the trained pathologist, that the College was well aware of the deplorable lack in this country of competent physicians skilled in clinical pathology and the inability of hospitals to secure such service. As against the old evil of assigning the laboratory to some staff physician with whom it was a side issue or to an interne who was more interested in surgical technic, it is far better to have a trained technician in charge. Adequate laboratory service, however, means the conduct of the laboratory under the direction of a clinical pathologist who, according to the definition of the American Society of Clinical Pathologists, is a physician who has had at least two years' experience in chemistry, bacteriology, serology, clinical microscopy and tissue diagnosis. It is a specialty which requires a preparation, training, code of ethics and ideals similar to what the American College of Surgeons demands of those aspiring to be Fellows.

Clinical pathology, therefore, should not be, as intimated in some quarters, a stepping-stone to internal medicine or surgery, howsoever valuable a knowledge of this may be in the practice of these branches, but an end in

*The phrase has since been changed to read "under competent supervision" which is interpreted by the official bulletin of the American College of Surgeons as being "best done through the medium of a clinical pathologist."

itself, a career, a life's work. The technic of a test may be learned easily even by the layman. The proper evaluation of the findings requires a broad knowledge of medicine. The exact diagnosis of pathologic tissue beset by so many pitfalls, requires years of experience. Hence the futility of entrusting this serious task to one who is looking forward to abandon it for some other and more lucrative field of activity.

And this brings us to the painful consideration of the crux of the problem, the economic or financial side of the laboratory, which one loathes to inject into this scientific gathering but which, alas, cannot be avoided. In this prosaic world adequate service, laboratory or what not, cannot be had without proper compensation. The present famine in pathologists for whom hospitals in the country are clamoring is solely due to the inadequate reward offered for their talents. The knowledge acquired by the clinical pathologist in fitting himself for his specialty forms a wonderful asset in the domain of surgery or of internal medicine, both of which beckon to him and lure him on with promise of wealth and fame. Even morally his status is often confounded with that of a mere technician. It is not surprising therefore that men of promise in clinical pathology gravitate to other specialties.

The solution to this question resides in a recognition of the fact that laboratory service should not be considered as something furnished by the hospital like room, board, light or heat, but as a medical consultation indispensable to the patient and paid for by him in accordance with his means.

This is not the occasion to enter into details of financing the hospital laboratory; suffice it to say that in our opinion it should be self-supporting. The income should be derived from charging the patients for the services rendered on a reasonable and equitable basis.

In view of the scarcity of pathologists and the necessity of their adequate compensation, it is expedient for one or more hospitals to engage the services of a directing pathologist who will spend the requisite time daily in each institution to supervise the work of the technicians, interpret the findings and make out the tissue diagnosis.

In this way may be solved the problem of the small hospital in the rural communities which are at present without laboratory facilities or incompetently served by a technician. A pathologist from the metropolitan center may visit these institutions in outlying towns at periodical intervals much in the way of a circuit preacher. He will control the work of the technician and diagnose the tissue sections. In addition the pathologist will serve as a stimulus to the medical staff in maintaining the program of standardization in other particulars, such as record keeping and staff meetings. The pathologist in furnishing adequate laboratory service is in the best position to keep up the scientific spirit of the institution as planned by the requirements of the American College of Surgeons.

His work should not stop with merely doing what is asked of him by the clinician. It is also a part of his function to initiate scientific work by tactful suggestion to the physician in casual interviews or at the monthly staff meetings. It is at the monthly gathering of the hospital staff that the pathologist

is called on to defend his diagnoses, which sometimes run counter to those of the surgeon. On these occasions he can advance the cause of scientific medicine by calling attention to new laboratory procedures in diagnosis of disease or in demonstrating interesting pathologic specimens from the operative material of the hospital and from autopsies. Particularly should the pathologist emphasize the necessity of postmortem examination and solicit the attending physician to secure permission, for the percentage of autopsies obtained is an index of the scientific status of the institution.

Lastly we must not omit the proper keeping of records in the laboratory as an essential to adequate service. Not only should a duplicate of all reports be kept in proper books or card index but a classification of diagnoses made on tissue sections should be maintained for ready reference. One or two slides of each section should be carefully catalogued and preserved for future reference. A monthly report of the activities of the laboratory should be presented at the staff meetings.

In the manner above outlined, the laboratory personnel and the pathologist can best be of service to the clinician and exemplify the spirit of standardization of doing the greatest good to the patient.

THE OCCURRENCE OF PARATYPHOID AGGLUTININS IN SPECIMENS SUBMITTED FOR THE TYPHOID AGGLUTINATION TEST*

By Ruth Gilbert, M.D., and William M. Groesbeck, B.S.†

THE clinical course of typhoid fever is so variable that similarity of the symptoms to those of a surprisingly large number of other diseases may at times cause a confusion in diagnosis. At least two other members of the typhoid-colon group of bacilli, namely, paratyphoid bacilli A and B, are the etiologic factors in typhoid-like diseases, often considered clinically almost identical with typhoid fever. Hence, it has been the custom in this laboratory to make a routine examination for paratyphoid agglutinins on all specimens of blood submitted for the typhoid agglutination test.

In the six years, from 1918 to 1923, during which such examinations have been made, 13,759 specimens of blood have been received for the typhoid agglutination test. The results obtained upon one hundred and fifteen of these have been excluded from the present study, since only tests for typhoid agglutinins were made. Thus, the actual number upon which paratyphoid tests were made is 13,644.

Thirteen thousand and forty of these specimens were tested, as a routine procedure, in the 1:40 dilution of the blood with *B. paratyphosus A* and *B* and if a reaction was obtained the test was repeated in the three dilutions,

*Presented at the meeting of the New York State Association of Public Health Laboratories, held in Rochester, New York, April 22, 1924.

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Received for publication, May 28, 1924.

1:20, 1:40 and 1:80. The results, summarized in Table I, have been divided into those in which complete agglutination occurred and those in which a partial agglutination reaction was observed (i.e., partial in 1:40 dilution).

TABLE I

RESULTS OF ROUTINE TESTS FOR PARATYPHOID AGGLUTININS

YEAR	TOTAL SPECIMENS TESTED	COMPLETE AGGLUTINATION		PARTIAL AGGLUTINATION	
		NUMBER	PER CENT	NUMBER	PER CENT
1918	2794	0	–	0	–
1919	2228	1	0.05	3	0.13
1920	2143	6	0.28	44	2.05
1921	2495	3	0.12	24	0.96
1922	1838	0	–	2	0.11
1923	1542	2	0.13	18	1.16
Total	13040	12	0.09	91	0.7

An average of 0.09 per cent (approximately 1 in 1000) routine agglutination tests has shown complete agglutination of paratyphoid bacilli and 0.7 per cent (7 in 1000) has shown partial agglutination.

Six hundred and four specimens were tested for paratyphoid agglutinins at the request of the physician or because the histories indicated that paratyphoid fever was suspected. The results of these tests are given in Table II.

TABLE II

RESULTS OF TESTS FOR PARATYPHOID AGGLUTININS WHERE PARATYPHOID INFECTION WAS INDICATED

YEAR	NUMBER OF SPECIMENS TESTED	COMPLETE AGGLUTINATION		PARTIAL AGGLUTINATION	
		NUMBER	PER CENT	NUMBER	PER CENT
1918	102	5	4.9	4	3.9
1919	85	3	3.5	0	–
1920	96	0	–	9	9.4
1921	134	2	1.5	8	6.0
1922	99	2	2.0	3	3.0
1923	88	0	–	8	9.0
Total	604	12	2.0	32	5.3

An average of 2.0 per cent (20 in 1000) of these Tests has shown complete agglutination and an average of 5.3 per cent (53 in 1000) has shown partial agglutination.

Conclusion.—The infrequency with which the presence of paratyphoid agglutinins has been demonstrated indicates that the test for them may be omitted from the routine examination of specimens submitted for the typhoid agglutination test without materially detracting from the value of these examinations. Whenever paratyphoid infection is indicated, however, the test for the presence of these agglutinins should be made.

THE PHARMACOLOGY OF BENZYL ALCOHOL AND ITS ESTERS

IV. The Diuretic Effect of Benzyl Alcohol, Benzyl-Acetate and Benzyl-Benzoate

By Charles M. Gruber, Ph.D., M.D., St. Louis, Mo.*

MACHT[1] studied the toxicity of the benzyl esters, also their effect upon kidney function on dogs. He found that prolonged administration of benzyl-benzoate and benzyl-acetate, both by mouth and by injection produced no chronic intoxication. Examination of the urine indicated no impairment of the kidneys, and functional tests with phenolsulphonephthalein showed no change in kidney function.

While testing the effect of oral administration of the benzyl esters upon the alimentary canal in dogs[2] it was noted that there was frequent urination in addition to frequent vomiting and defecation. At first this action was thought to be due to the relaxation of the bladder sphincter and the copious drinking of water. However, in a later series of experiments[3] with the intravenous administration of benzyl alcohol, benzyl-acetate and benzyl-benzoate these substances were found to produce circulatory changes within the kidneys. These facts suggested that it would be interesting to note if the above substances had an effect on the secretion of urine.

METHOD

Dogs and rabbits were employed. The former animals were anesthetized either with paraldehyde 1.8 ml. per kg. by mouth or with ether by inhalation. Urethane, 2 gm. per kg. body weight, by stomach or ether by inhalation, were used for the latter animals. The rabbits were fed generous quantities of green cabbage several days before the experiments were performed.

The blood pressure, when recorded, was always taken from the left carotid artery with a mercury manometer. A 2 per cent sodium citrate solution was employed as an anticoagulant. A chronometer indicating either 5, 15 or 30 second intervals, was employed. When the blood pressure was recorded the chronometer also indicated the zero blood pressure level.

Cannulas were placed in each ureter and the drops of urine secreted were recorded on the kymograph by receiving and recording tambours.

Benzyl alcohol, benzyl-acetate and benzyl-benzoate were used in 10 per cent emulsions made with 5 per cent acacia in Ringer's solution.[3] These were injected slowly into the lateral ear vein of the rabbit. The pure preparations were used for intramuscular and intraperitoneal injections. In some experiments the intestine was ligated near the pyloric sphincter.

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Received for publication, May 28, 1924.

RESULTS

Intravenous Administration.—The results upon intravenous administration were not satisfactory. In many instances the rate of urine flow was markedly diminished and in some cases there was complete suppression. The results were influenced by the quantity and rate of injection of the drug. The suppression appeared to be secondary to the effect of the drug upon the heart muscle, respiratory system and blood pressure. See Gruber.[3] The greater the fall in blood pressure the greater the suppression of urine. In some instances intravenous injections were followed by marked augmentation in the rate of urine flow. Fig. 1, which was obtained from a 3 kg. rabbit, illustrates the normal flow of urine taken as control. At X^1 2.5 ml. 5 per

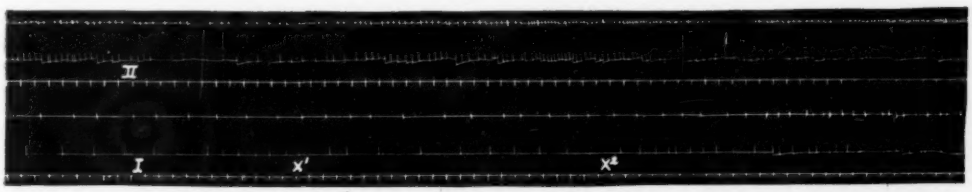

Fig. 1.—Rabbit 3 kg. Urethane anesthesia. Cannulas in both ureters. Upper record left kidney, middle record right kidney secretions in drops. I. Control rate of urine flow in drops. At X^1 2.5 ml. acacia solution five per cent and at X^2 2.5 ml. acacia solution five per cent containing ten per cent benzyl-benzoate were injected intravenously; II. Continuation of record I. Time in thirty seconds. This and all following illustrations are reduced ⅓ or more.

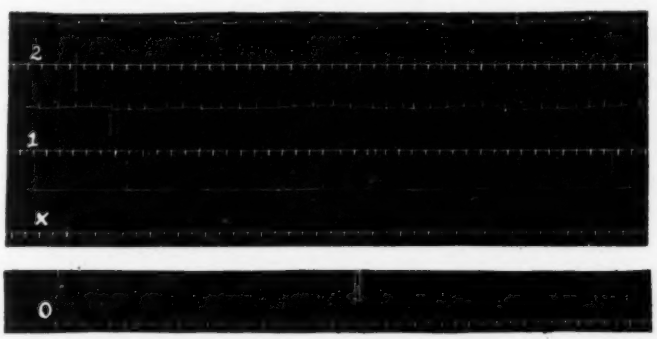

Fig. 2.—Rabbit, urethane anesthesia. Cannula placed in left ureter near the hilus of the kidney. *0*, control rate of urine flow for thirty-seven minutes. X, 1 ml. pure benzyl alcohol was injected intramuscularly; 1, fifty minutes later; 2, one hundred minutes after X. Time in thirty second intervals.

cent acacia was injected into the lateral ear vein. There was produced no noticeable change in urine flow. At X^2 the same amount and concentration of acacia but containing 10 per cent benzyl-benzoate was injected intravenously. Within six minutes the urine flow was augmented. It was increased from about 1 drop per minute to about 8 drops per minute for each kidney. This augmented urine flow continued for ninety minutes longer than shown in the figure.

Intramuscular Administration.—That diuresis can be produced by these drugs when they are injected intramuscularly can be seen in Fig. 2. In this animal the cannula was placed in the left ureter near the hilus of the kidney. The normal rate of urine flow was recorded for thirty-seven minutes, of

which curve 0 is an example, before the injection was made. At X, 1 ml. pure benzyl alcohol was injected intramuscularly; curves 1 and 2 were taken respectively fifty and one hundred minutes later. In this animal the effect of the drug was shown in eight minutes. The maximum rate of urine flow was 2 drops per minute, whereas before the injection the rate was 1 drop per three minutes.

Intraperitoneal Administration.—The most satisfactory results were obtained by the administration of pure preparations of benzyl alcohol, benzyl-acetate and benzyl-benzoate intraperitoneally. The degree of increased urine flow and the duration of the latent period varied with the different animals used. In nearly all instances the duration of the latent period was shortest for benzyl alcohol but longest for benzyl-benzoate. This will be seen upon comparing Tables I, II and III. The average time of the latent period for benzyl alcohol in 22 animals thus studied was found to be about seven minutes, for benzyl-acetate in 10 animals eight minutes, and for benzyl-benzoate in 7 animals nineteen minutes.

In a previous communication[4] the author found no evidence to support Nielsen and Higgins'[5] claim that the different benzyl esters varied in their rate of action upon the blood pressure. They maintain that there was a difference and that this difference in rate of action was dependent upon the

TABLE I

TABLE SHOWING THE APPROXIMATE TIME INTERVAL BETWEEN THE TIME OF INJECTION AND THE BEGINNING OF AUGMENTATION IN URINE FLOW AFTER BENZYL ALCOHOL WAS INJECTED INTRAPERITONEALLY. MAXIMAL RATE OF FLOW OF URINE IN DROPS SHOWN IN TABLE

ANIMAL	WEIGHT IN KILOGRAMS	PURE BENZYL ALCOHOL IN ML.	TIME OF LATENT PERIOD IN MINUTES	MAXIMAL RATE OF URINE FLOW IN DROPS PER MINUTE			
				RIGHT KIDNEY		LEFT KIDNEY	
				NORMAL	AFTER BENZYL ALCOHOL	NORMAL	AFTER BENZYL ALCOHOL
Rabbit	2.0	1.0	10	...	...	0.5	2.5
	3.0	1.5	12	0.3	4.3	0.3	3.0
	3.2	1.6	7	0.7	5.3	...	...
	3.0	1.0	3	0.3	3.0	0.2	3.0
	5.0	2.5	3.5	3.0	5.3	2.5	5.5
	4.3	2.2	3	...	...	1.0	4.0
	3.9	2.0	3	1.0	4.0	...	...
	3.6	2.0	5	2.0	4.0	...	...
	3.7	2.0	14	0.3	1.7	...	...
	3.4	2.0	10	1.0	2.0	1.0	1.7
	3.9	2.0	4	2.7	5.0	4.7	7.3
	2.5	1.5	6	...	...	0.3	2.8
	2.5	1.0	3	4.0	6.5	4.0	6.0
	3.2	1.0	2	1.0	3.0	0.5	1.5
	3.0	1.0	20	0.5	1.5	0.3	0.8
	3.0	1.0	16	1.0	1.3	1.2	1.8
	2.3	1.0	1	1.5	6.5	1.2	6.5
	4.3	1.0	14	1.8	6.0	2.5	5.5
Dog	18.0	3.0	5	2.8	3.8	3.5	4.5
	14.5	5.0	7	1.8	2.0	..	...
	9.5	2.5	6	0.5	8.5	1.0	8.5
	14.1	1.0	2	2.0	4.0	2.0	5.5
Average			7.1	1.5	4.1	1.55	4.1
Increase in per cent					173		170

respective rates of hydrolysis. The above findings support Nielsen and Higgins' view that these esters may be converted into their components before producing their action. In this connection it must also be remembered that the rate of absorption from the peritoneal cavity will vary with their respective solubilities. Inasmuch as benzyl alcohol and benzyl-acetate are fairly soluble and benzyl-benzoate practically insoluble in water, one would naturally expect some difference in their rates of action.

The average increased rate of urine flow is slightly greater for benzyl-benzoate than for either benzyl-acetate or benzyl alcohol, it being about 215, 182 and 171 per cent respectively. The average increase was 190 per cent for the entire series of experiments.

Benzyl Alcohol.—Benzyl alcohol has a decided action upon the kidney function. See Figs. 3, 4 and 5 and Table I. Figs. 3 and 4 were obtained from experiments upon rabbits. It will be seen that in Fig. 3 the blood pressure

TABLE II

TABLE SHOWING THE APPROXIMATE TIME INTERVAL BETWEEN THE TIME OF INJECTION AND THE BEGINNING OF INCREASED URINE FLOW AFTER BENZYL-ACETATE WAS INJECTED INTRAPERITONEALLY. MAXIMAL RATE OF FLOW OF URINE IN DROPS SHOWN IN TABLE.

ANIMAL	WEIGHT IN KILOGRAMS	PURE BENZYL-ACETATE IN ML.	TIME OF LATENT PERIOD IN MINUTES	MAXIMAL RATE OF URINE FLOW IN DROPS PER MINUTE			
				RIGHT KIDNEY		LEFT KIDNEY	
				NORMAL	BENZYL-ACETATE	NORMAL	BENZYL-ACETATE
Rabbit	2.5	1.0	14.0	1.0	2.4	1.0	2.4
	3.0	1.3	7.5	2.0	4.0	...	...
	3.9	2.0	5.0	1.4	3.4	1.4	3.7
	3.4	2.5	10.0	0.3	1.3	...	...
	2.3	1.0	4.0	1.0	3.0	1.0	5.8
	2.3	1.0	6.0	0.5	3.0	0.5	3.0
	3.8	2.0	4.0	0.5	6.0	0.3	6.0
Dog	11.0	5.0	15.0	2.5	3.0	1.3	1.5
	15.0	5.0	10.0	2.5	3.0	2.0	2.5
	11.0	4.0	8.0	0.3	0.8	0.3	0.8
Average			8.3	1.2	3.0	1.0	3.2
Increase in per cent					150		220

TABLE III

TABLE SHOWING THE APPROXIMATE TIME INTERVAL BETWEEN THE TIME OF INJECTION OF BENZYL-BENZOATE INTRAPERITONEALLY AND THE BEGINNING OF INCREASED URINE FLOW. MAXIMAL FLOW OF URINE IN DROPS SHOWN IN TABLE

ANIMAL	WEIGHT IN KILOGRAMS	PURE BENZYL-BENZOATE IN ML.	TIME OF LATENT PERIOD IN MINUTES	MAXIMAL RATE OF URINE FLOW IN DROPS PER MINUTE			
				RIGHT KIDNEY		LEFT KIDNEY	
				NORMAL	BENZYL-BENZOATE	NORMAL	BENZYL-BENZOATE
Rabbit	2.1	0.5	25	0.5	3.0	0.5	2.0
	2.4	1.2	18	3.0	21.0	2.0	9.0
	3.0	1.5	45	1.0	5.0	1.0	6.0
	5.25	1.0	8	2.5	6.0	1.5	5.3
	3.0	1.0	4	8.0	12.5	5.8	8.3
Dog	10.3	5.0	23	1.5	3.5	1.5	2.5
	14.5	5.0	10	1.3	6.5	1.0	6.5
Average			19	2.5	8.2	1.9	5.7
Increase in per cent					228		200

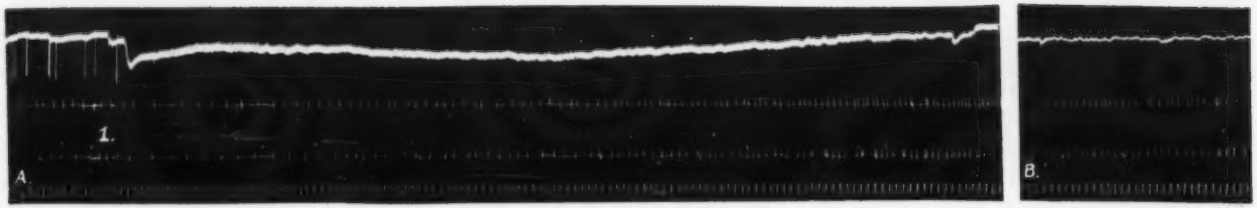

Fig. 3.—Rabbit 4.3 kg. Urethane anesthesia. Top record is blood pressure, bottom record is time in 15 second intervals and zero blood pressure. Of the remaining records the upper is the rate of secretion of urine in drops from the right kidney and the lower from the left kidney. In this and the remaining records cannulas were placed in the ureters. *A*, 1. Intraperitoneal injection of 1 ml. pure benzyl alcohol. *B*, Record taken thirty minutes after terminus of *A*.

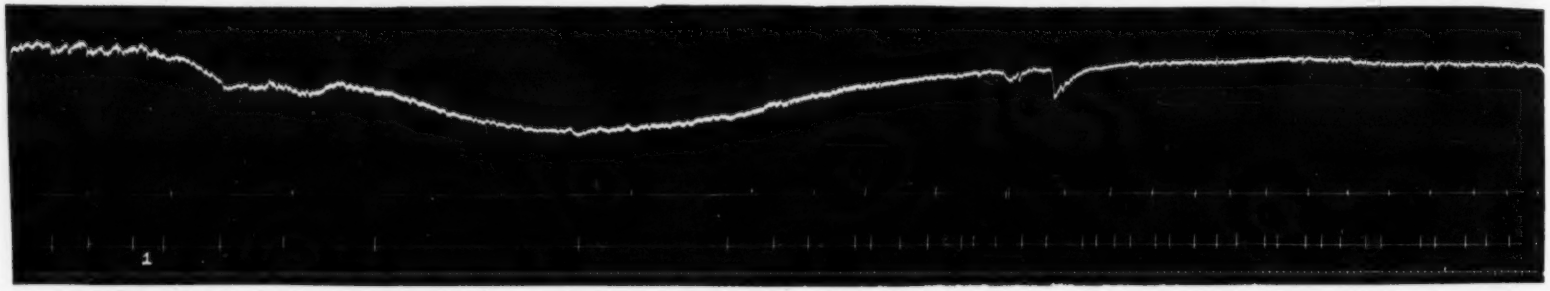

Fig. 4.—Rabbit 3 kg. Urethane anesthesia. Top record is the blood pressure, the bottom one is the time in fifteen second intervals and zero blood pressure. Of the remaining records the upper is the rate of secretion of urine in drops from the left kidney and the lower from the right kidney. 1. Intraperitoneal injection of 1 ml. pure benzyl alcohol.

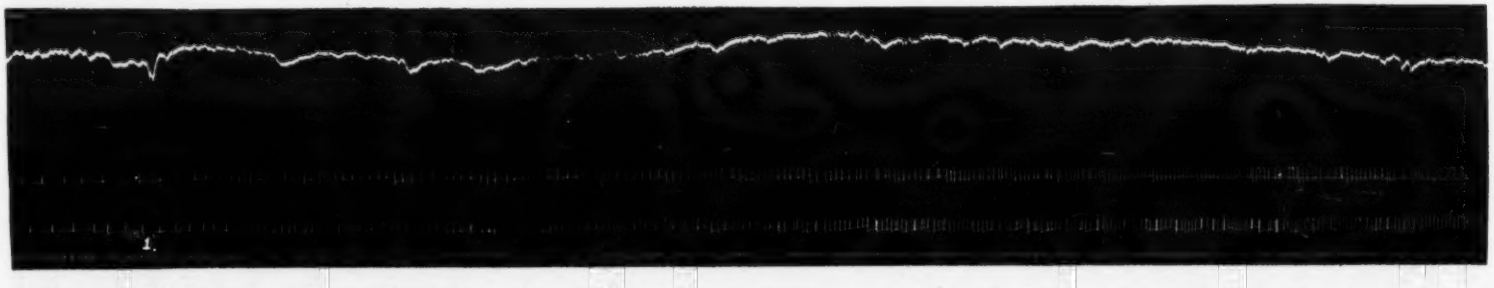

Fig. 5.—Rabbit, 2.3 kg. Urethane anesthesia. Same as Fig. 4. Record continued for forty-five minutes at which time the blood pressure dropped to 84 mm. of mercury and the secretion of urine was 1.5 drops for the left and 2 drops for the right kidney per minute.

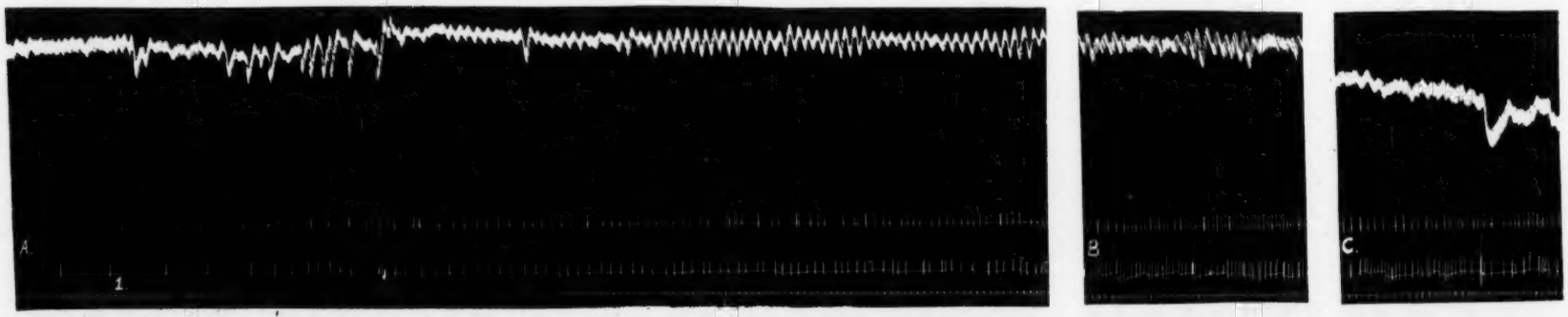

Fig. 6.—Dog, 9.6 kg. Paraldehyde anesthesia. Same as Fig. 4 except as follows: *A*, 1, 2.5 ml. pure benzyl alcohol were injected intraperitoneally; *B*, fifteen minutes after end of *A*; *C*, thirty minutes after end of B.

fell after the intraperitoneal injection of 1 ml. pure benzyl alcohol. This sudden fall in pressure is accompanied by a slowing in the secretion of urine. Following this there is a more gradual fall in blood pressure, then a return to normal with a marked increase in the secretion of urine. In some instances, as illustrated in Fig. 4, the blood pressure fell to such an extent that the secretion of urine was almost stopped. In this instance, however, the blood pressure returned to normal and the urine secretion went above normal. In some other animals the blood pressure remained low during the remaining length of the experiment and anuria resulted.

In Fig. 5 the blood pressure did not fall after the injection of 1 ml. pure benzyl alcohol. It increased from 122 to 135 mm., which later returned to 116 mm. of mercury. Simultaneously with this change, the number of drops of urine excreted increased from 1.5 drops per minute to 6 drops for the left kidney and to 7 drops for the right kidney. That benzyl alcohol and its esters when injected intravenously can increase the blood pressure was shown by the author in a previous communication.[3] These results upon intraperitoneal administration further support the finding given there as do the observations obtained on the dogs used in securing Figs. 6 and 9.

Intraperitoneal injection of 2.5 ml. of pure benzyl alcohol was made in a dog weighing 9.6 kg. in Fig. 6. The blood pressure temporarily fell from 160 to 150 mm. of mercury but ten minutes after the injection it had risen to 170 mm. of mercury, falling gradually to 160 mm. during the next thirty minutes. Thirty minutes later, or seventy minutes after the injection, the blood pressure was only 110 mm. of mercury. As seen in B in Fig. 6 the secretion of urine increased rather rapidly, reaching a maximum forty-five minutes after the injection. Although the blood pressure dropped more than 50 mm. in C, nevertheless the rate of urine flow is very much faster than the control rate in A.

Benzyl-acetate.—Benzyl-acetate like benzyl alcohol has a short latent period. This is seen in Table II and Fig. 7. After the injection of benzyl-acetate in Fig. 7 the blood pressure gradually fell from 120 to 76 mm. of mercury. The excretion of urine rapidly increased and continued above the normal rate for more than two hours. Like benzyl alcohol, benzyl-acetate occasionally produced a rapid fall in blood pressure accompanied by anuria, from which the animals did not recover during the time of the experiment.

Benzyl-benzoate.—Figs. 8 and 9 are presented to show the effects of benzyl-benzoate upon the rabbit and dog respectively. In Fig. 8, curve 1 shows the normal rate of secretion of urine by both kidneys; 2 the injection of 1.2 ml. pure benzyl-benzoate intraperitoneally; and 3 and 4 the rate of secretion of urine fifty and one hundred minutes respectively after the injection. The rate of secretion before the injection was about 1 drop per minute which reached a maximum rate of 3 drops per minute after the injection. In this animal the blood pressure was not recorded.

The dog used in Fig. 9 weighed 14.5 kg. and received 5 ml. pure benzyl-benzoate intraperitoneally at 1. The blood pressure rose as the record shows from 120 to 142 mm. of mercury but later fell to 120 mm., curve C. After

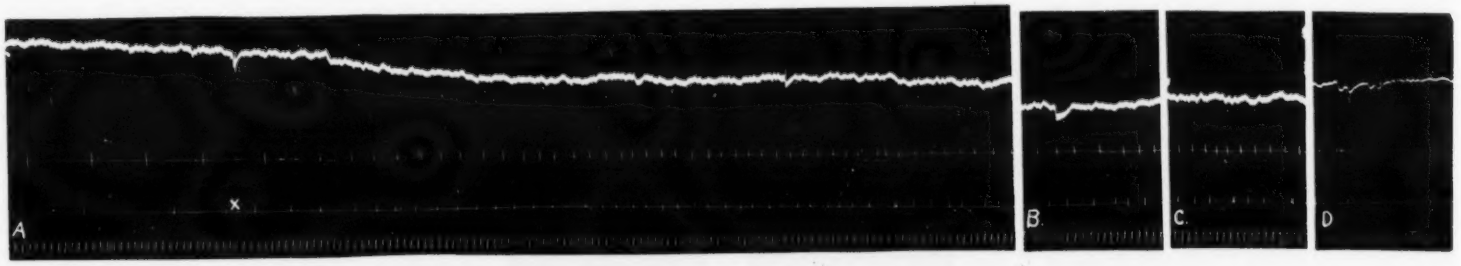

Fig. 7.—Rabbit, 3.8 kg. Urethane anesthesia. Same as Fig. 3, except as follows; X, 2 ml. pure benzyl-acetate were injected intraperitoneally; *B*, thirty minutes after end of *A*; *C*, one hour after *B*; and *D*, thirty minutes after *C*.

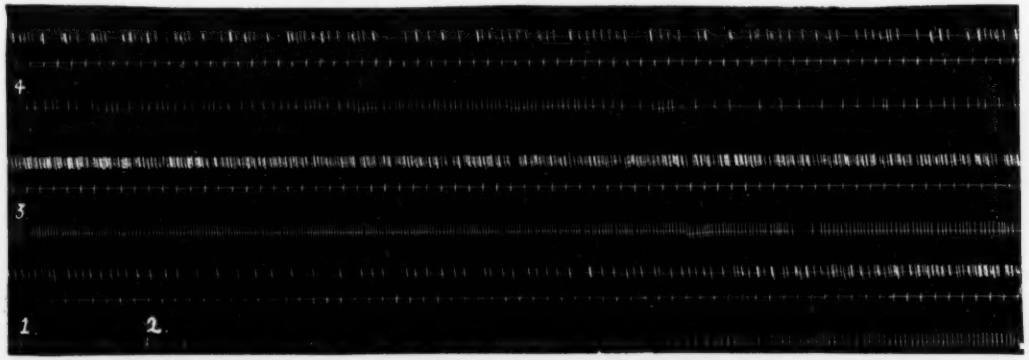

Fig. 8.—Rabbit 2.4 kg. Urethane anesthesia. Urethane given one hour before operation. Top record secretion of urine in drops by right kidney, middle time in thirty second intervals, bottom record secretion of urine in drops by left kidney. 1. Control rate. 2. Intraperitoneal injection of 1.2 ml. pure benzyl-benzoate. 3 and 4. Records taken fifty and one hundred minutes respectively after the injection.

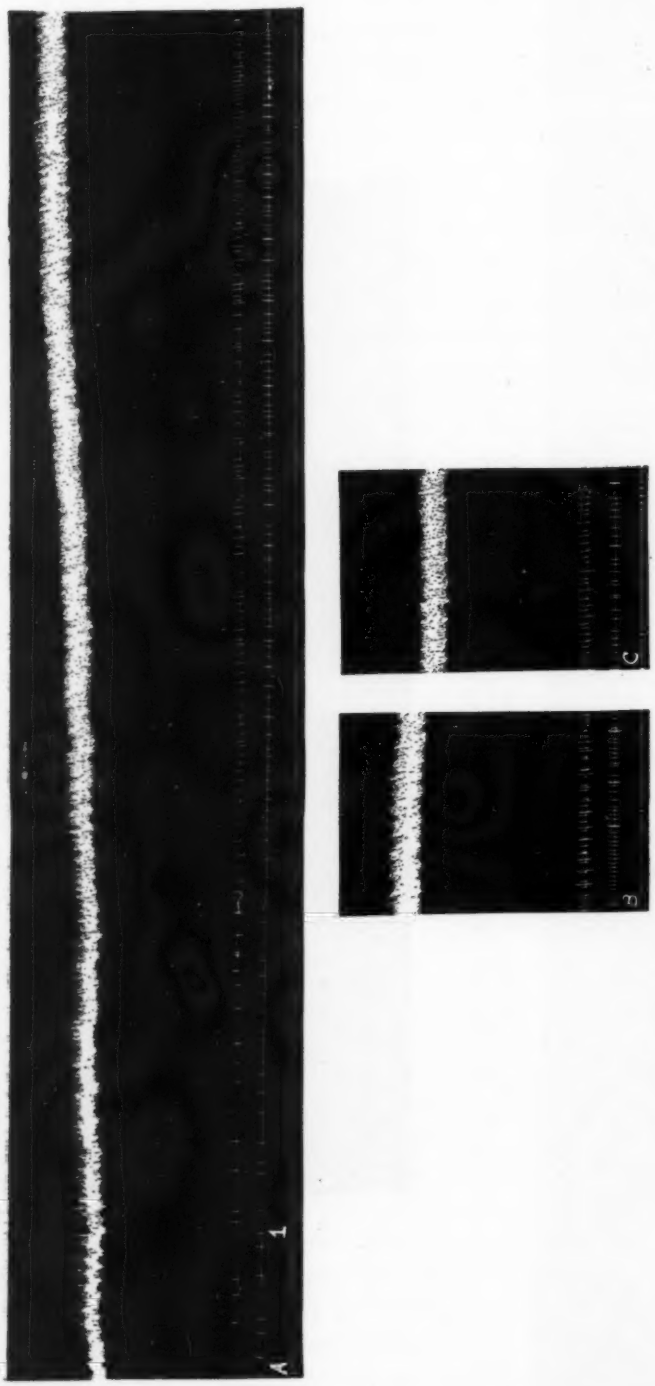

Fig. 9.—Dog, 14.5 kg. Paraldehyde anesthesia. Same as Fig. 6, except as follows; at 1, 5 ml. of pure benzyl-benzoate were injected intraperitoneally; *B*, fifteen minutes after *A*; *C*, twenty-five minutes after *B*.

the injection the rate of urine flow gradually increased from 1 and 1.3 drops to 6 and 6.3 drops for the left and right kidneys respectively.

DISCUSSION

From the above results it will be seen that benzyl alcohol and its esters benzyl-acetate and benzyl-benzoate increase the rate of urine secretion. Inasmuch as diuresis occurred even after pyloric sphincter ligation it cannot be due to relaxation of these smooth muscles thus permitting rapid emptying of the fluid contents of the stomach into the intestine. In a previous communication[2] plethysmographic records were shown demonstrating a decreased volume of the kidney in all the animals tested. In some instances the blood pressure was not changed nevertheless the kidney decreased in volume while at the same time the intestine increased in volume[3] (Fig. 9). Perfusion experiments confirmed Macht's[1] finding however, that the kidney vessels may dilate under the influence of benzyl alcohol and its esters.[3a] Whether benzyl alcohol and its esters irritate the kidney cells to further secretion, as is thought to be the case with calomel[6]; change the glomerular membrane so that fluids are filtered more readily, as some authors think to be the case with caffein[7]; decrease the rate of reabsorption of fluids in the tubules, as is thought to be the case with sodium sulphate[8]; increase the number of glomeruli secreting as has been found to be the case with caffein by Richards and Schmidt[9]; or increase intrarenal arterial blood pressure and increase volume flow through the kidney, cannot be stated at this time. Experiments are now being performed in an effort to determine if possible the mode of action of benzyl alcohol and its esters in producing this increased urine secretion. Our findings on the action of these drugs upon urine secretion support those of Macht's,[10] upon the circulation to the effect that rabbits are much more susceptible to the benzyl esters than are dogs. We found rabbits to respond by a much greater increase in the rate of urine flow than the dogs used in the experiments.

SUMMARY

1. Benzyl alcohol and its esters benzyl-acetate and benzyl-benzoate cause increased urine secretion when given either intravenously, intramuscularly or intraperitoneally.

2. The latent period between the intraperitoneal injection and beginning of increased urine secretion in 22 animals was found to be seven minutes with benzyl alcohol; eight minutes in 10 animals with benzyl-acetate and nineteen minutes in 7 animals with benzyl-benzoate.

3 Benzyl alcohol was found to increase the rate of urine flow 171 per cent in 22 animals, benzyl-acetate 182 per cent in 10 animals and benzyl-benzoate 215 per cent in 7 animals, an average of 190 per cent for the entire series.

4. The point of action of the drug has not been determined.

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THE PREPARATION OF A SATISFACTORY POIKILOTHERMOUS ANIMAL*

BY ROBERT W. KEETON, M.S., M.D., CHICAGO, ILL.

IN the course of an investigation into heat regulation it became necessary to prepare poikilothermous animals. This loss of the power to maintain a constant body temperature in the face of changing environmental temperatures results when the cervical cord of an animal is transected. Many of the investigators (see ref. 1-6) who have prepared such animals have subjected them to experimental studies within a short time (3 to 4 hours) of their recovery from the anesthetic. However, my experiments required that the animals recover from the immediate effects of the operation and return to their normal feeding routine. Freund and Strassman[7] have reported the most complete experiments on poikilothermous animals surviving for a number of days. Many of their rabbits lived from seven to ten days. They record, however, a rather high mortality occurring within the first twenty-four hours postoperative. These deaths were attributed to surgical shock, but no attempt was made to analyze further the factors involved. Since this type of animal is useful in physiology for teaching and demonstration purposes and offers an opportunity for the study of the vasomotor mechanism, it seemed advisable to present a technic which has proved successful in its preparation.

Selection of Animal.—In the preparation of a cervical cord animal, the rabbit offers some advantages over the dog. The phrenic nerve in the rabbit[8] arises chiefly from the fourth segment with small branches from the third and fifth. In the dog[9] it arises from the fifth, sixth and seventh cervical nerves. It is evident that the chances of damaging the phrenic nuclei are much greater in the dog than the rabbit. After section of the cord the motility of the intestines is increased[10] so that a diarrheic state readily establishes itself. With the rabbit this may be controlled by the character of the food. It never becomes troublesome. In the dog because of the frequent respiratory tract infections (sniffles, distemper) and this increased intestinal motility, the stools rapidly become watery, foul smelling, and streaked with blood. The use of bone ash in the food may help but it does not eliminate entirely

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Received for publication, July 20, 1924.

this bowel disturbance. Bed sores never develop in the rabbit. They always occur in dogs with high section if the animals live long enough. It is however quite difficult to maintain the body weight of the rabbits. They are helpless and as a result have to be fed with the hand. They eat slowly so that almost constant attention must be given to their feeding over a period of two to three hours daily, if anything like an adequate quantity of food is eaten.

Preparation of the Operative Field.—Rabbits readily develop staphylococcus infections. Such infections have been observed occurring spontaneously in the mammary glands of two otherwise healthy animals and in the axillary space of a third. If tincture of iodine be used for the sterilization of the field of operation, a slough will appear in a few days along the wound edges. The organisms invade this slough and if the animals be sacrificed at varying intervals following the operation the progress of this infection may be traced from the surface to the spinal cord. The application of 70 per cent to 95 per cent alcohol to the skin gives satisfactory antisepsis. It does not cause a slough and its use eliminates this type of wound infection.

Anesthetics and Heat Loss.—The operation can be performed under ether but the hazard is great. Rabbits die so readily under a simple ether anesthetic that it requires a definite period of training before an assistant can be regarded as a safe anesthetist. Then the ether predisposes to pneumonia an animal which at best is very susceptible to this form of infection. However, the important objection to ether lies in the very rapid loss of heat after section of the cord. Moore[11] has observed that the administration of ether to a normal rabbit for an hour may lower the rectal temperature 1.5° C. Our experience shows that within a short period following the transection of the cord a lowering of the temperature of 1° to 4° C. occurs. This fall was observed by the earlier investigators and was the subject of extended study. More recently their observations have been reviewed and summarized by Schönborn.[4] In my experience this fall occurs despite the protection of the animal by blankets and the application of external heat. Five to six hours are required to return the animal to a temperature of 38° C. and the rabbit normal (38.8°-39.4°) may not be reached for twelve hours.

If a local anesthetic (2 per cent novocaine) be used, the temperature may rise immediately after the section. In one case a temperature of 41° was noted and in two others a smaller elevation occurred. This recalls to mind the clinical experience that lesions of the cervical cord are often associated with hyperpyrexia. However, such rises are transitory and if the animal be not protected from heat loss the temperature will fall progressively. In the majority of cases there is a drop of 1° to 1.5° C. within twenty minutes of the section. If external heat be applied the temperature remains stationary or rises rapidly to normal. This is in sharp contrast to the prolonged lag present in the ether animals before the depression can be removed. The ether certainly exaggerates those changes in the heat regulating mechanism induced by cervical section.

Establishment of Reflex Block.—In four consecutive animals operated under deep surgical anesthesia, respirations ceased simultaneously with the section of the cord. In other animals the cord was cut under light ether anesthesia. They also stopped breathing with the completion of the section. This cessation of the respiration may have been due to a primary reflex respiratory inhibition or to a secondary one due to some form of acute circulatory failure. The injection of morphine 1.65 mg. and atropine 0.22 mg. in rabbits weighing 2 to 2.5 kilos eliminated this reflex factor when ether was employed.

In the use of novocaine, it was customary to give two injections of morphine (1.65 mg.) and atropine (0.22 mg.) at an interval of one hour. An even larger dose may be used with safety. After the exposure of the cord, a novocaine block was established. Not over 0.3 to 0.4 c.c. of 2 per cent novocaine should be injected. A larger quantity will establish an excellent block, but the animals quite frequently die one to three hours later. It has been assumed that death was due to an interruption of the synapses on the phrenic nuclei which lie in the immediate proximity.

The Immediate Blood Pressure Changes.—It is a well established experimental fact that section of the cord in this region deprives the animal of a compensatory mechanism which is active in maintaining blood pressure in the face of adverse hydrostatic conditions. Thus elevation of the feet causes a maintained rise in blood pressure, while lowering of the feet causes a similar fall. This is in sharp contrast to the behavior of the normal animal, which after a short delay in each set of experimental conditions returns the pressure to the normal level. Therefore, it is important that the feet be elevated before the cord is cut and that this relative position be maintained for a number of hours after the operation. On several occasions, when ether was being used as an anesthetic, it was found that the lifting of the head and shoulders of the animal, even for the purpose of bandaging, caused an almost immediate respiratory stand-still. It then became a rule not to remove an animal from the operating board or change his position for from twelve to eighteen hours after the section.

Treatment of Collapse after Cord Section.—Despite the use of precautions, which have been outlined above, a state of collapse, in which the respirations were labored and shallow and the tissues cyanotic, sometimes followed immediately upon section of the cord. If now an injection of 22 mg. of caffein sodium benzoate be given into the ear vein, the respirations become more rapid, the color of the tissues improves and in a short time the animal's condition is satisfactory.

Period of Experimental Usefulness.—The animals drink water in eight to twelve hours and begin to eat food within twenty-four hours in satisfactory quantities, and in normal quantities after forty-eight hours. On the third day they can be regarded as in good experimental condition. In this condition they remain for six to fourteen days. Emaciation of the paralytic portion of the body is rapid in spite of apparently satisfactory feeding. This wasting has been noted by other observers. It is probably largely due to inadequate feeding. The animal in his helpless condition can easily eat greens of all

types, but it is difficult to devise a method of feeding him oats and similar foods which are more nutritious. A nasal discharge manifests itself as the forerunner of a terminal pneumonia. This pneumonia is less apt to occur if the animals be operated upon immediately on their entrance to the laboratory before they have had opportunity to become infected from the stock rabbits. The last animal operated on was in good condition after twenty days and was sacrificed at this time. The autopsy showed complete section of the cord and no pneumonia.

Technic in Detail.—A rabbit (weight 2 to 2.5 kilos) is injected intramuscularly with morphine (1.6 mg.) and atropine (0.22 mg.). One hour later the

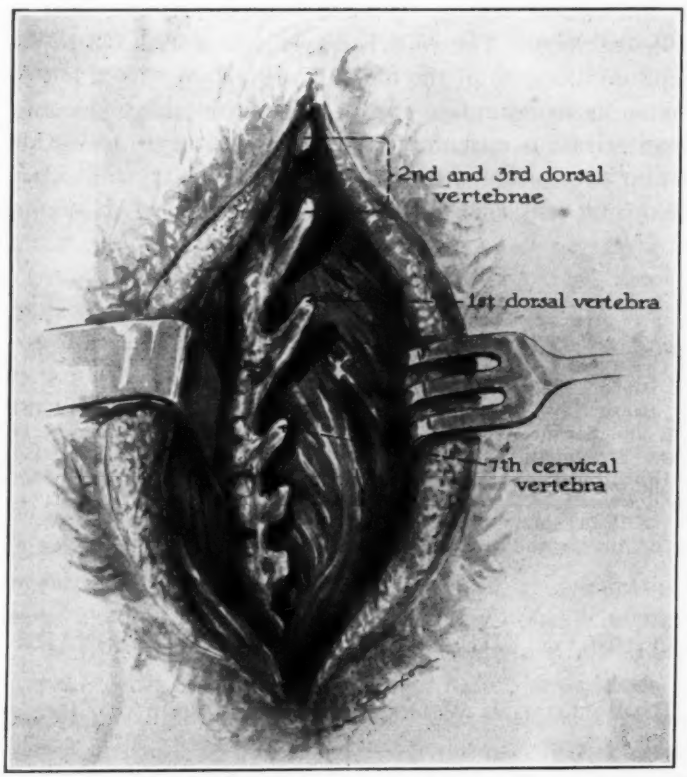

Fig. 1.

injection is repeated. Thirty minutes later (one and one-half hours after the first injection) the operation is begun. In the meantime the field has been shaved and prepared with alcohol. The animal is wrapped in a light blanket, placed on the left side with hind feet tied, front feet loose and the ears held by an assistant. The foot of the board is elevated four inches. Towels may now be clipped to the skin without any display of pain by the rabbit.

The skin is infiltrated with 2 per cent novocaine and the muscle layers blocked off by plunging a fine caliber needle to the lateral spinous processes. This catches the nerves shortly after their exit. For the entire operation 5 to 10 c.c. of the solution is usually sufficient. On exposing the spines of the

vertebrae, care should be taken to avoid the space lateral to the seventh cervical and the first dorsal vertebra. The vertebral vein is located here and it is quite easily damaged. A hemorrhage established may rapidly prove fatal. The seventh cervical spine may be located as follows. (See illustration.) The first two spinous processes which are long and of equal height are the second and third dorsal. The vertebra cephalad is about three-fourths as high and is of course the first dorsal. The next one marks the beginning of the cervical cord. Its height is so variable that it cannot be used as an identifying criterion. The spine is best removed by the use of a pair of sharp-pointed medium heavy scissors. These enable the operator to take short bites on either side of the process. If too much of the dorsum of the vertebra is removed, the intraspinal venous plexuses may be injured with a resulting rapidly fatal hemorrhage. The cord is now blocked with 0.3 to 0.4 c.c. novocaine. Immediately the area of the block is cauterized with a hot probe. This is done slowly so as to coagulate the tissues within the block and avoid extending the cauterization into unanesthetized territory. It is this extension which appears to precipitate the collapse (shock) in the animals. After the closure with catgut, external heat is applied as needed to maintain the temperature.

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FRACTIONATION OF THE PROTEINS OF RATTLESNAKE VENOM*

By William H. Welker, A.C., Ph.D., Chicago, Ill.

IN 1904, John Marshall[1] announced that he had succeeded in separating the proteins of rattlesnake venom into toxic and nontoxic fractions. Up to the time of his announcement, none of the investigators had succeeded in getting nontoxic protein fractions from the venom. The principle of the method employed was the addition of saturated ammonium sulphate solution in such a manner that the concentration of ammonium sulphate in no part of the solution was ever markedly increased beyond that of any other part. This was accomplished by employing a dilute solution of the venom and very rapid mechanical stirring while adding saturated solution of ammonium sulphate.

*From the Laboratory of Physiological Chemistry of the College of Medicine of the University of Illinois, Chicago, Illinois.
Received for publication, July 21, 1924.

Marshall was compelled to abandon further experimentation through accidental sensitization to the venom of the rattlesnake which has persisted to the time of the writing of this article. In transferring some newly acquired rattlesnakes from the box in which they had been transported, one of the snakes in the box struck and momentarily entangled its fangs in the wire netting covering of the box and ejected venom, some of which came in contact with one of Marshall's eyes. Intense perspiration within five minutes of the occurrence was the first symptom observed; the conjunctiva became decidedly distended with an extravasation of a yellowish serum which disappeared in about three days on treatment with cold compresses. This accident, however, resulted in a sensitization so marked that dried venom, or indeed a solution of venom, left exposed in the same room produced an intense disturbance in his air passages with the production of great quantities of a thick, tenacious mucus, the disturbance lasting two or three months.

In a personal communication to the writer, Marshall pointed out that the only way to obtain rapid and complete solution of the venom is to moisten the dried material with a few drops of one per cent sodium chloride solution and when completely softened to dilute with one per cent sodium chloride solution to the desired volume. If the venom shall not have been filtered, preferably through a very small, hardened filter paper, immediately after its procurement from the snake, a quantity of material in the dried venom, consisting of debris from broken down cellular tissue, will refuse to dissolve in any liquid. Pulverizing the venom renders a very large portion of the substance insoluble.

Marshall, in his communication, also states that in his experiments, the autopsies on the pigeons in which death occurred from the injection of the toxic fraction, showed the spleen intensely congested. The intestinal tract also was congested but to a less degree than the spleen.

Experimental.—In the work reported herewith, an apparatus was devised for purposes of stirring which could be driven by a small motor. In Experiment I, it was endeavored to redissolve the individual fraction and reprecipitate the same until the solution of the fraction showed no precipitation on either side of the limits of the fraction. This entailed very serious loss of protein material, because of a marked tendency of a given fraction to shift its precipitation limits. On account of this difficulty, the number of reprecipitations for the different fractions was reduced in Experiment II.

Experiment I.—Ten grams of dry rattlesnake venom were moistened with a small quantity of physiologic salt solution and when complete liquefaction had been effected, the solution was made up to one liter with distilled water and permitted to stand twenty-four hours under toluol. A white flocculent precipitate resulted. This method of precipitation was used for euglobulin because of the very gradual formation of the precipitate. The white flocculent precipitate was filtered off, dissolved in 10 per cent sodium chloride solution and precipitated by a saturation up to 33 per cent of ammonium sulphate. The precipitate was redissolved and reprecipitated in this fashion four times, including the first precipitation, then dissolved in a 10 per cent sodium chlo-

EXPERIMENT ON GUINEA PIGS

PROTEINS OF FRACTIONATION EXPERIMENT I

INJECTIONS MADE INTRAPERITONEALLY

	FRACTION	WEIGHT OF SUBSTANCE	WEIGHT OF GUINEA PIGS	REMARKS
A	(0 to 33 per cent)	10 mg.	270 gm.	No effect
B	(33 to 46 per cent)	10 mg.	300 gm.	No effect
C	(46 to 64 per cent)	10 mg.	350 gm.	No effect
D	(64 to 100 per cent)	10 mg.	365 gm.	No effect
E	(about 100 per cent)	5 mg.	235 gm.	No effect

Solutions of all fractions but A (0 to 33 per cent) were made in physiologic salt solution, and fraction A was made in 5 per cent sodium chloride solution in which it was only slightly soluble.

EXPERIMENT ON PIGEONS

PROTEINS OF FRACTIONATION EXPERIMENT I

INJECTIONS MADE INTO THE BREAST MUSCLES OF PIGEONS

	FRACTION	WEIGHT OF SUBSTANCE	WEIGHT OF PIGEONS	REMARKS
A	(0 to 33 per cent)	10 mg.	333 gm.	*
B	(33 to 46 per cent)	10 mg.	327 gm.	No effect
C	(46 to 64 per cent)	10 mg.	339 gm.	No effect
D	(64 to 100 per cent)	10 mg.	344 gm.	No effect
E	(above 100 per cent)	5 mg.	313 gm.	No effect

The solutions of these fractions were made in the same way as in the experiment on guinea pigs.

*No effects were noted except in the case of Fraction A. This pigeon did not appear abnormal until fourteen hours after the injection and was found dead, in rigor mortis, twenty-five hours after the injection.

ride solution, filtered and dialyzed free from ammonium sulphate and sodium chloride, centrifugalized and washed with distilled water until the washings gave no biuret test.

The 33 to 46 per cent fraction was precipitated four times including its initial precipitation. The solution of the third precipitate showed practically no precipitation at 33 per cent and practically none at 46 per cent of ammonium sulphate solution. The solution of the fourth precipitate was filtered and dialyzed free from ammonium sulphate and sodium chloride.

The 46 to 64 per cent fraction was precipitated three times, then dissolved and dialyzed free from ammonium sulphate and sodium chloride.

The saturated filtrate from the first precipitation of the albumin was dialyzed free from ammonium sulphate and sodium chloride.

Experiment II.—In a second experiment, two and one-half grams of dry rattlesnake venom were moistened with a small amount of physiologic salt solution and when complete liquefaction was effected, the solution was diluted to 250 c.c. with distilled water and permitted to stand overnight. The precipitate was filtered off, dissolved in 10 per cent sodium chloride, saturated to 33 per cent of ammonium sulphate, filtered, washed by centrifugalization with distilled water until free from soluble protein, and dried.

The 33 to 46 per cent fraction was precipitated from the filtrate of the first fraction by saturation to 46 per cent of ammonium sulphate solution. The precipitate was dissolved in water, the solution saturated to 33 per cent, permitted to stand overnight and the precipitate which formed filtered off.

EXPERIMENT ON PIGEONS
PROTEINS OF FRACTIONATION EXPERIMENT II
INJECTIONS MADE INTO THE BREAST MUSCLES OF PIGEONS

	FRACTION	WEIGHT OF SUBSTANCE	WEIGHT OF PIGEONS	REMARKS
*1	(0 to 33 per cent)	10 mg.	290 gm.	No effect
2	(33 to 46 per cent)	10 mg.	295 gm.	Pigeon showed signs of discomfort 4½ hours after injection: found dead, but warm, seven hours after injection
†2A	(33 to 46 per cent)			
3	(46 to 64 per cent)	10 mg.	300 gm.	Pigeon recumbent 4½ hours after injection: found dead, but still warm 7 hours after injection
‡3A	(0 to 33 per cent)	10 mg.	320 gm.	No effect
4	(64 to 100 per cent)	10 mg.	295 gm.	No effect
5	(above 100 per cent)	10 mg.	300 gm.	No effect

Solutions made in physiologic salt solution, except in the case of 3A, which was made in 10 per cent sodium chloride.

*Insoluble in 10 per cent salt solution.
†From the solution of the first precipitate at (46 to 64 per cent) saturation.
‡From the solution of the first precipitate at (33 to 46 per cent) saturation. Insoluble in water, soluble in 10 per cent sodium chloride solution.

EXPERIMENT ON PIGEONS
PROTEINS OF FRACTIONATION EXPERIMENT II
INJECTIONS MADE INTO THE BREAST MUSCLES OF PIGEONS

	FRACTION	WEIGHT OF MATERIAL	WEIGHT OF PIGEONS	REMARKS
2A	(33 to 46 per cent)	10 mg.	297 gm.	Dead three hours after injections
3	(46 to 64 per cent)	10 mg.	317 gm.	Recumbent in five minutes after injection, gasping for breath 13 minutes after, dead 14 minutes after injection
3A	(0 to 33 per cent)	10 mg.	318 gm.	Slight signs of discomfort 15 minutes after injection, later normal
*4A	(46 to 64 per cent)	10 mg.	327 gm.	No effect
5	(above 100 per cent)	10 mg.	292 gm.	No effect

Solutions made in physiologic salt solution except in the case of 3A which was made in 10 per cent sodium chloride solution.

*Precipitate up to 64 per cent saturation of ammonium sulphate from second precipitation of albumin.

The filtrate was raised to 46 per cent saturation with ammonium sulphate solution. The precipitate was filtered off, dissolved and dialyzed.

The precipitate at 33 per cent of saturation was washed with 33 per cent saturated ammonium sulphate solution, dissolved in 10 per cent sodium chloride and dialyzed free from salts. The suspension of the precipitate was centrifugalized, the precipitate washed with distilled water by centrifugalization until free from soluble protein and dried. This material is indicated as "3-A" in the animal experiments.

The filtrate from the first precipitate of the 33 to 46 fraction was saturated to 64 per cent and the precipitate was filtered off, redissolved in water and saturated to 46 per cent. The precipitate at 46 per cent was filtered off, dissolved in water and dialyzed free from ammonium sulphate and is indicated as "2-A" in the animal experiments. The filtrate was saturated to 64 per cent with ammonium sulphate solution. The precipitate was dissolved in water and the solution dialyzed free from ammonium sulphate.

The filtrate from the 64 per cent precipitate was saturated with chemically pure, solid ammonium sulphate. This precipitate was dissolved in distilled water and the solution saturated to 64 per cent. The precipitate occur-

ring at this degree of saturation was filtered off, dissolved, dialyzed and dried. This fraction is indicated as "4-A." The filtrate was saturated with solid ammonium sulphate, the precipitate filtered off, dissolved and dialyzed free from ammonium sulphate and sodium chloride.

All the solutions of the precipitates were dried at 40° C. in the presence of toluol.

The saturated solution of ammonium sulphate was dialyzed free from ammonium sulphate for the purpose of securing any colloidal material not precipitated by full saturation with ammonium sulphate.

DISCUSSION OF RESULTS

In the toxicity tests made on products of the first fractionation experiment, it appears that practically all of the toxicity had been lost from the protein fractions. The fraction from 46 to 64 per cent which was found toxic by Marshall and which also showed high toxicity in the second experiment is nontoxic when purified by reprecipitations from its accompanying toxic principle. The slight toxicity of the euglobulin fraction can be explained on the basis of incomplete removal of water-soluble protein by washing. While the wash water showed no biuret-reacting substances, the fact that this fraction showed slight solubility after drying, would indicate that not all of the water-soluble material had been removed from it.

In the toxicity tests from the materials of the second fractionation experiment, the only fractions showing toxicity were first: the fraction precipitated by a saturation of 46 to 64 per cent and the 2A fraction which was the precipitate occurring between 33 and 46 per cent saturation in the first reprecipitation of the 46 to 64 fraction. In this experiment the euglobulin fraction when dried at 40° C. (after water-soluble proteins and electrolytes had been removed) became completely insoluble in 10 per cent sodium chloride. A similar observation has been made in this laboratory, in the fractionation of blood serum in another investigation which will be published shortly. Rippel, Ornstein, Carl, and Lasch[2] have recently reported a similar observation.

CONCLUSIONS

1. The results of these experiments confirm the work of Marshall in showing the feasibility of separating rattlesnake venom into toxic and nontoxic protein fractions.

2. They show that the euglobulin on being dried after the removal of water-soluble proteins and electrolytes is no longer soluble in 10 per cent sodium chloride.

3. There is an indication that on repeated reprecipitation of the toxic fraction, the toxic principle contained in the fraction may be removed.

Further work is in progress on the toxic fraction, with a view of studying the nature of the toxic substances of the venom.

My thanks are due Professor Marshall for his kind advice and suggestions in connection with this investigation and also for the rattlesnake venom used in the investigation.

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LABORATORY METHODS

QUANTITATIVE WASSERMANN TEST*

By E. H. Ruediger, M.D., Los Angeles, Calif.

I BELIEVE that the Wassermann test is here to stay for some time to come and consider it our duty to develop it to the highest degree of efficiency. In order to develop it intelligently we must agree on some sort of a unit of measure and do strictly quantitative work. Without a unit of measure quantitative work cannot be done, and without quantitative work we do not get the information we ought to have, and we do not give the clinician and the patient the information for which they long and which they should obtain.

Many laboratories are still doing no more than qualitative work with the Wassermann test. They never report more than 4+ (++++). This means at least 4+, but the serum tested may titrate 50+, 100+ or even 1,000+. Supposing that a patient who would give 1,000+ result with the Wassermann test and has a report which says 4+ or ++++, is put on treatment and is treated for a year and the reports on the Wassermann test still read 4+, the patient and the physician are sure to be discouraged because it shows no improvement. Entirely different is the situation when quantitative work is done. If the first report reads 1,000+, treatment is urgently indicated, and if the second report, about six months after beginning of treatment reads 400+, patient and physician receive encouragement.

If the third report, about a year after the first report, reads 200+ patient and physician receive new encouragement, moreover they will soon understand that under such conditions negative results are not to be expected within a few months, and frequently treatment must be continued for several years, and that many chronic cases never become negative.

For several years I have been doing quantitative titration on every serum that was submitted, and the results have been very satisfactory. The following report shows some of the results obtained on cases that could be followed up for some time.

METHOD

Human Serum.—All human sera were heated to about 56° C. for thirty minutes and were mixed with pure, sterilized glycerol so as to have a mixture containing fifty per cent glycerol. The first dilution I usually make 1:4 by adding one part of human serum to three parts of a mixture containing two

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Read before the Third Annual Convention of the American Society of Clinical Pathologists, Rochester, Minnesota, June 7, 1924.

parts of glycerol and one part of physiologic salt solution. (I mix 25 c.c. of physiologic salt solution with 50 c.c. of glycerol and add 0.3 c.c. of the diluted glycerol to every 0.1 c.c. of human serum used. Glycerol so diluted is easily handled with a pipette and the resulting dilution of the human serum is 1:4.) I prefer to heat the serum before adding the diluted glycerol. Any further diluting is done with 50 per cent glycerol in physiologic salt solution.

Antigen.—As so-called antigen I employ plain alcoholic extract of human heart muscle or of beef heart muscle and use it in the optimum dilution and dose. This must be determined for each lot of antigen.

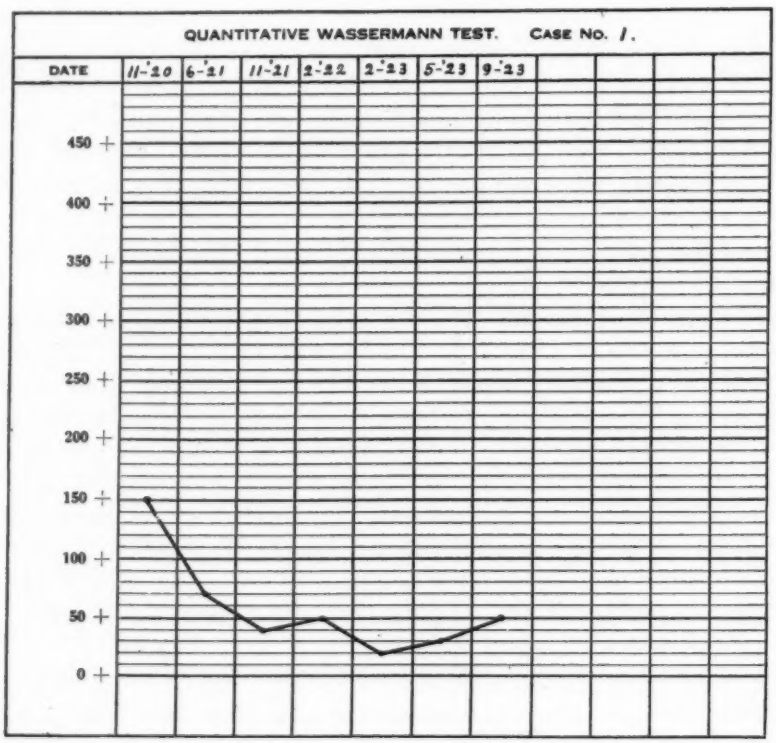

Chart 1.

Complement Serum.—All complement sera are selected. Each complement serum is separatively, quantitatively titrated against a known negative control serum and against a known positive control serum, and all complement sera that are not satisfactory are rejected.

Hemolytic System.—The hemolytic system may be human or sheep; I use human, simply because it is much easier to get human corpuscles. In the precision method I use 1 hemolytic unit, and in the routine method I use 1.5 hemolytic unit.

The Test.—For the routine test put six suitable tubes into a suitable test tube rack, and designate the tubes as Nos. 1, 2, 3, 4, 5 and 6. Let tube No. 1 be the control tube and tubes Nos. 2, 3, 4, 5 and 6 the antigen tubes. Into each of tubes Nos. 3, 4, 5 and 6 put 0.2 c.c. of 50 per cent solution of glycerol. Into each of the first two tubes put 0.2 c.c. of human serum

diluted 1:4 and into the third tube put 0.1 c.c. of the human serum diluted 1:4. Mix the contents of tube No. 3 and transfer 0.1 c.c. to tube No. 4; mix and transfer 0.1 c.c. to No. 5; mix and transfer 0.1 c.c. to No. 6; mix and discard 0.1 c.c. The dilutions will be as follows, control tube 1:4, antigen tubes 1:4, 1:12, 1:36, 1:108, 1:324. These are the dilutions I mostly employ, but the dilutions may be multiplied by 2, 4 or 5 without greatly changing the final results. Add 0.2 c.c. of physiologic (0.9 per cent) salt solution to the control tube and 0.2 c.c. of diluted antigen to each of the antigen tubes and shake. Add 0.2 c.c. of complement serum diluted 1:7.5 to each tube and shake.

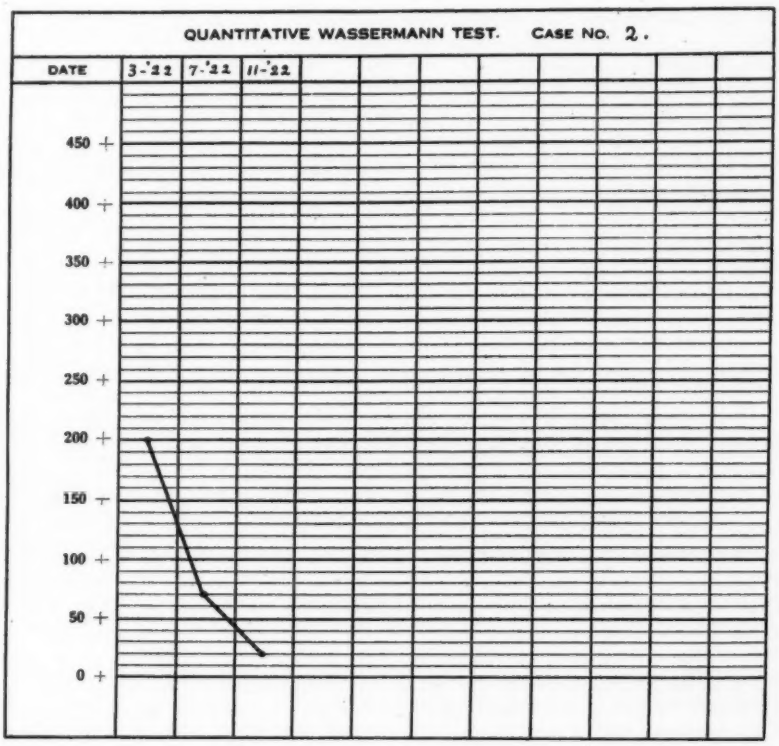

Chart 2.

Place all tubes in ice-cold water for five hours shaking them well about two hours after they have been put into the cold water.

Wash human blood corpuscles and make a 2.5 per cent (1:40) suspension of well-packed corpuscles in physiologic salt solution.

Dilute the hemolytic amboceptor with physiologic salt solution.

Titrate the hemolytic system and adjust it to 1 unit with 0.2 c.c. of complement serum diluted 1:10.

At about thirty minutes before the hemolytic amboceptor and corpuscles are to be added to the test mix equal parts of diluted amboceptor and corpuscle suspension, shake well, put the mixture into the incubator at about 37° C. for thirty minutes, shaking at intervals of ten minutes.

Transfer the tubes from the cold water-bath to a warm water-bath at 37° C. and from five to ten minutes later add 0.4 c.c. of amboceptor-corpuscle mixture to each tube. Return the tubes to the warm water-bath for one hour during which time they should be shaken at intervals of fifteen minutes. Remove the tubes from the warm water-bath, let them stand at room temperature for about one hour, and read the results.

Reading Results.—In reading results complete inhibition of hemolysis in a tube is called 1+ or one unit fixation and this is multiplied by the dilution of the human serum.

Example.—

No. of Tube		1	2	3	4	5	6
Dilution of Human Serum		1:4	1:4	1:12	1:36	1:108	1:324
No. of Test							
1	Reading	0	+	+	+	0	0=36+
2	"	0	+	+	+	+	0=108+
3	"	0	+	+	+	+	+=324+

If the last antigen tube does not show the limit of fixation the serum is titrated further, beginning with a higher dilution.

Example.—

No. of Tube		1	2	3	4	5	6
Dilution of Human Serum		1:100	1:100	1:200	1:400	1:800	1:1600
No. of Test							
1	Reading	0	+	+	+	0	0=400+
2	"	0	+	+	+	+	0=800+

The following report shows the results obtained on seven syphilitic patients who were under observation for varying lengths of time.

CASE 1.—This was a chronic syphilitic who came under observation in November, 1920. The first Wassermann test, with blood obtained in November, 1920, gave 150+. A second Wassermann test, in June, 1921, gave 70+. In November, 1921, the Wassermann test showed 40+, in February, 1922, it showed 50+, in February, 1923, it showed 20+, in May, 1923, it had gone up to 30+ and in September, 1923, it had risen to 50+. See Chart 1.

CASE 2.—This was a child about ten years old who had congenital syphilis. At this time the parents sought treatment because the eyes of the child had become very bad. The first Wassermann test was done in March, 1922, and showed 200+. In July, 1922, the Wassermann test showed 70+ and in November, 1922, it was 20+. See Chart 2.

CASE 3.—A child about eight years old had a very bad throat which did not respond to ordinary treatment. The first Wassermann test was done in April, 1922, and showed 500+. Antisyphilitic treatment was given and the throat improved rapidly. In July, 1922, the Wassermann test showed 400+, in October, 1922, it showed 300+ and in January, 1923, it showed 200+. In this case the objective and subjective symptoms concerning the actual lesion disappeared rapidly, and the quantitative Wassermann test showed decided improvement, but an old-fashioned qualitative Wassermann test might have shown ++++ with each of these four tests. See Chart 3.

CASE 4.—Patient 4 of this series was a child about seven years old with congenital syphilis who was brought for treatment because he had become nearly blind. The first Wassermann test was done in July, 1922, and showed 400+. The second test was done in October, 1922, and was 40+; by April, 1923, it had risen to 100+ again, and in September, 1923, it was found to be 20+. See Chart 4.

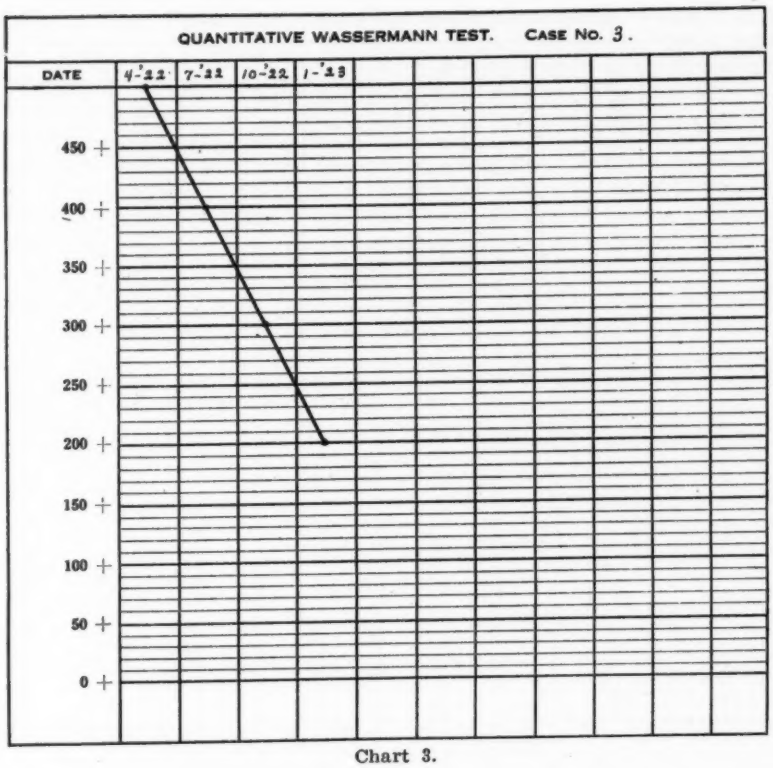

Chart 3.

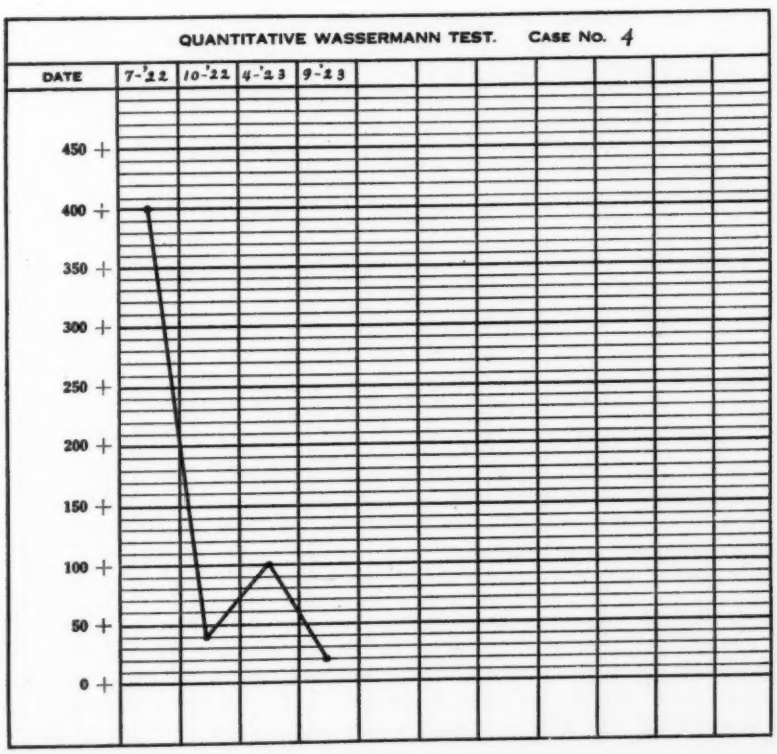

Chart 4.

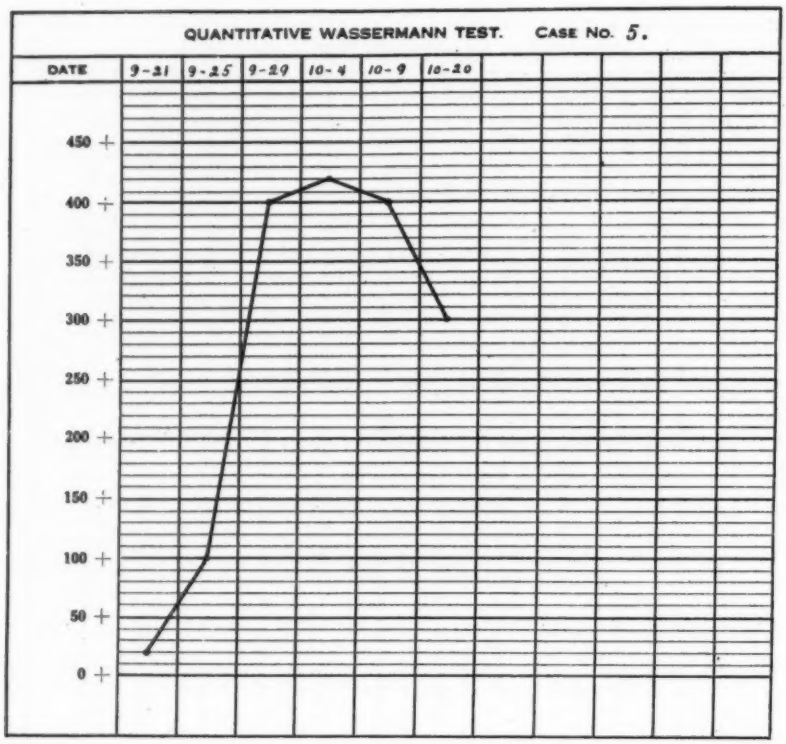

Chart 5.

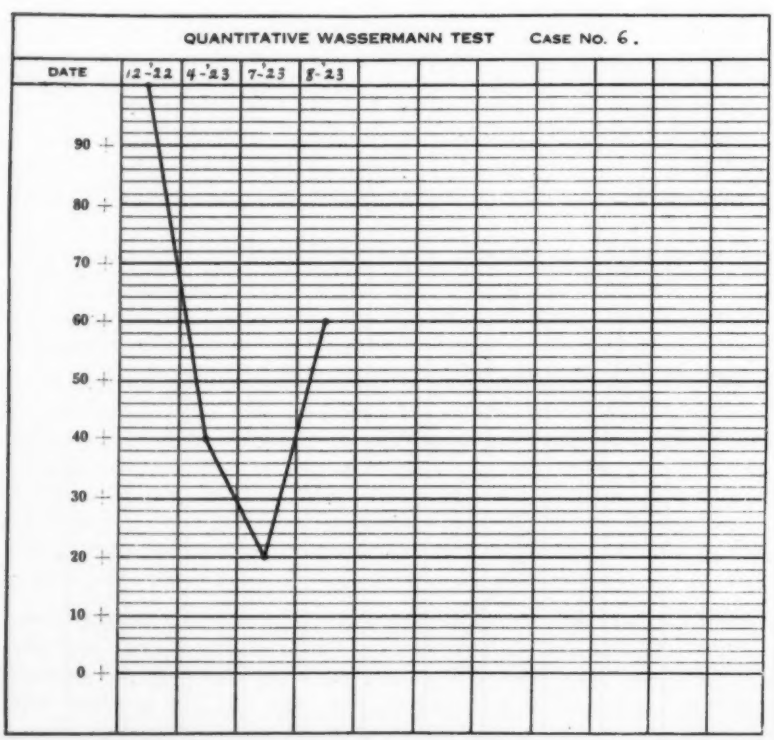

Chart 6.

CASE 5.—Case No. 5 is an extremely interesting one. A young man consulted a physician because of a venereal sore which had appeared a few days before. The physician convinced himself that the lesion was not a syphilitic lesion, gave the patient something to be used locally and asked the patient to come back after a few days. The patient returned and the sore was bigger than it had been at the first visit. After reprimanding the patient for not having used the medicine, the physician sent him to the hospital where a Wassermann test was done on September 21, and was found to be 20+. This did not change the physician's views; he still considered the present lesion nonsyphilitic. On September 25 a second Wassermann test was done and this one showed 100+, but without making any impression on the physician because he had excluded syphilis. On September 29, the Wassermann test showed 400+, the patient had a profuse purple rash and antisyphilitic treatment was begun. The rash faded rapidly and the sore began to heal. Wassermann tests done on October 4, October 9, and October 20, showed 420+, 400+, and 300+ respectively. See Chart 5.

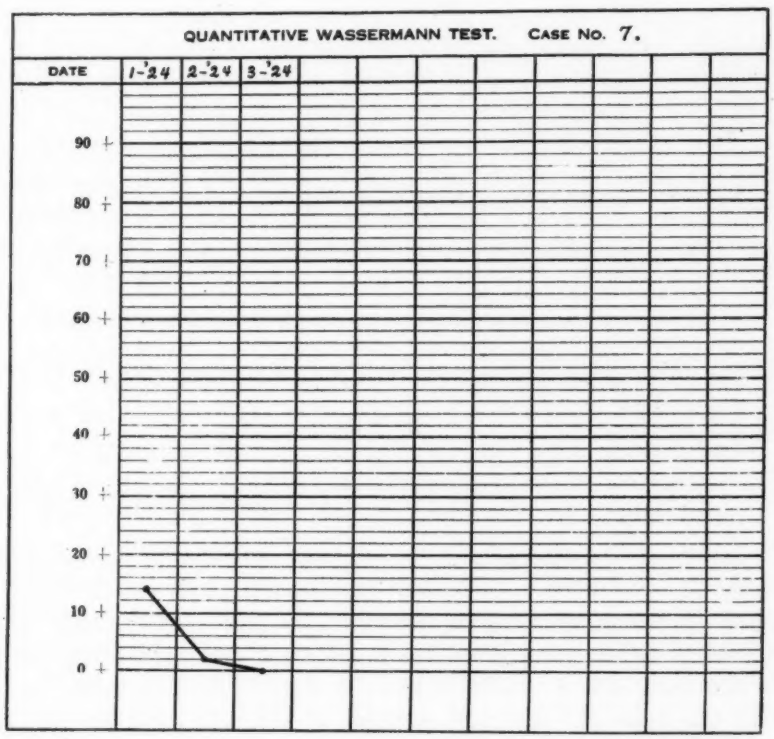

Chart 7.

CASE 6.—A woman consulted her physician because she was rapidly losing her hearing. A Wassermann test was done in December, 1922, which showed 100+. She was given antisyphilitic treatment. In April, 1923, the Wassermann test showed 40+; by July, 1923, it had fallen to 20+; treatment was discontinued and the patient was instructed to return within a month. In August, 1923, the Wassermann test showed 60+ and the patient was advised to take treatment regularly for some time to come. See Chart 6.

CASE 7.—This case demonstrates the importance of showing improvement even with the Wassermann test. A young man contracted syphilis, went to his physician and was put on antisyphilitic treatment. The first Wassermann test at that time was reported as strongly positive, ++++. The treatment was taken regularly and Wassermann tests were done at intervals of a few months but the reports on them always read "strongly positive,

++++.'' The patient became disgusted with this report and went to another physician for further examination and treatment. The second physician asked me to do a Wassermann test on this patient in January, 1924, and I reported, ''positive, 12+,'' and explained the difference between qualitative and quantitative Wassermann test. The patient was well pleased, he received no treatment but was instructed to return in a month. In February, 1924, he reported for another Wassermann test which was done and found to be 2+. This time the patient was greatly pleased; he received no treatment but was instructed to report for another Wassermann test after one month. In March 1924, I did the third Wassermann test on this patient and found it negative. This patient has been instructed to come here for Wassermann test once or twice a year to find out whether it remains negative or becomes positive again, and some time in the future I may be able to report on this case again. See Chart 7.

SUMMARY

Quantitative titration in the Wassermann test is as satisfactory as quantitative titration of typhoid agglutinating serum, diphtheria antitoxin or tetanus antitoxin. A quantitative Wassermann test gives far more information than does a qualitative Wassermann test. In early doubtful cases a rising titre with the Wassermann test may be considered diagnostic of syphilis. During treatment, a falling curve with the Wassermann test gives valuable information and encouragement to patient, physician and laboratory worker.

(*For discussion, see p.* 303.)

A NOTE ON THE PREPARATION OF COLLOIDAL GOLD SOLUTION BY THE MELLANBY-ANWYL-DAVIES TECHNIC

By Russell L. Haden, M.D.*

THE preparation of colloidal gold is an unsatisfactory procedure for many laboratories. The technic of Miller, Brush, Hammers and Felton,[1] which is employed by most workers, is tedious and time consuming. Different batches of the solution, even when made the same day, will often vary quite widely in reaction. Good results in the test depend largely upon the correct reaction of the reagent. An acid solution is too sensitive, and an alkaline one is not sensitive enough.

Mellanby and Anwyl-Davies[2] have recently described a new method of preparation which is very simple. They state that the solutions so made are always neutral in reaction. The solutions that I have prepared by their technic, however, have always been too acid, so that a luetic type of curve is obtained even with a normal spinal fluid. This result is probably due to a difference in the acid content of the gold chloride used by the English workers. In attempting to overcome this difficulty I have added potassium carbonate without very satisfactory results. Uniformly good solutions can be made, however, by the addition of potassium hydroxide. The optimum amount to be added can be determined by a preliminary test using the reagents which are to be employed in the preparation of the reagent.

*From the University of Kansas Medical School, Kansas City, Kansas.
Received for publication, July 2, 1924.

Reagents.—(1) One per cent gold chloride solution made by dissolving a 15 grain tube of Merck's gold chloride in 100 c.c. of freshly distilled water.

(2) One per cent potassium oxalate solution prepared by dissolving 1 gram of *neutral* potassium oxalate in 100 c.c. of freshly distilled water.

(3) One per cent solution of potassium hydroxide made by dissolving 1 gram of potassium hydroxide (c.p. purified by alcohol) in 100 c.c. of freshly distilled water.

Preliminary Test.—Set up six clean test tubes in a rack and into each run 1 c.c. of the 1 per cent gold chloride. Add 1 per cent potassium hydroxide as follows: Tube No. 1, 0.6 c.c., No. 2, 0.5 c.c., No. 3, 0.4 c.c., No. 4, 0.3 c.c., No. 5, 0.2 c.c., and No. 6, 0.1 c.c. To each tube add 2 drops of a 1 per cent solution of phenolphthalein in 95 per cent alcohol. The tube containing the largest amount of alkali will remain clear and react alkaline. The other tubes show varying degrees of turbidity, the one having the optimum reaction being the most turbid. This is usually the tube containing 0.3 c.c. alkali. One c.c. of 1 per cent neutral potassium oxalate is now added to each tube. Reduction will begin quickly, starting in the tubes having the optimum reaction. Within five minutes, tubes 4, 5 and 6 are completely reduced as indicated by a dense black precipitate; tube 1 is alkaline and clear; tube 2 is turbid and of a lead color due to partial reduction. The optimum amount of the potassium hydroxide to use in the preparation of the colloidal gold solution is the largest amount which can be added and still have complete reduction take place in five minutes as shown by the preliminary test. With the different samples of Merck's gold chloride which we have tried, this is usually 0.3 c.c. of the 1 per cent solution for each c.c. of the gold chloride solution. Colloidal gold solutions made with this amount of alkali are uniformly neutral. If only 0.2 c.c. is added the reagent is too acid, and with 0.4 c.c. it is too alkaline.

The amounts given may vary with different batches of gold chloride but the principle of correction is the same with all.

Preparation of Reagents.—The exact technic for the preparation of the reagent follows: To 100 c.c. of distilled water 1 c.c. of the 1 per cent potassium oxalate solution is added, and the fluid is heated to the boiling point. While boiling, 1 c.c. of the gold chloride solution, to which the requisite amount of alkali (i.e., a total of 1.3 c.c. if 0.3 c.c. is the optimum amount of alkali) has been added, is run in drop by drop. A clear red, neutral solution of colloidal gold is quickly obtained.

All glassware should be clean and of the hard variety such as Pyrex. The water must be doubly distilled, the second distillation being made immediately before use. Since city water supplies often contain chloride, it is preferable to start with spring or rain water. The stock solutions appear to keep indefinitely. It is an advantage to add 1 c.c. of 10 per cent potassium permanganate and 1 c.c. of a saturated solution of barium hydroxide to each 2 liters of water before the second distillation.

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[1]Miller, S. R., Brush, N. D., Hammers, J. S., and Felton, K. D.: Bull. Johns Hopkins Hosp., 1915, xxvi, 391.

[2]Mellanby, J., and Anwyl-Davies, T.: Brit. Jour. Exper. Path., 1923, iv, 132.

FURTHER STUDIES WITH CRESYLECHT VIOLET INCLUDING A REPORT OF MY SIX-SECOND METHOD FOR STAINING TISSUES*

By B. G. R. Williams, M.D., Paris, Illinois

Assisted by E. M. Williams, B.S., Paris, Ill., and Sr. M. Tiburtia, Terre Haute, Ind., and Miss Margaret Drummy, Terre Haute, Ind.

LET us see in fancy, a method for tissue staining suitable both to fresh and fixed materials, easily completed in six seconds and with the following reactions resultant, viz.:

Nuclei.—Dark blue.
Cytoplasm.—Light blue or violet.
Blood.—Pea green.
Muscle.—Greenish blue or blue (according to thickness of sections).
Fat.—Bright, translucent lemon-yellow or orange.
Fibrils.—Pink to magenta.
Fibrin.—Sky-blue.
Calcification.—Dirty blue.
Caseation.—Granular-blue.
Metachromatic reactions for mucin, colloid, hyalines, etc., etc.

Such a staining method should prove of great value, particularly in surgical pathology.

METHODS IN GENERAL USE

Various polychrome methylene blues have been used. Their shortcomings have been many. They will in a general way, stain nuclei blue and stroma a bluish-red; but metachromasia stops there. The blues stain fairly rapidly but not sharply, giving only general outlines and little detail. They aid in some diagnoses; but in no manner approach the results suggested above. Of these blues, the best in my opinion, is that of Goodpasture; that of Wilson is also with some merits while that of Terry is more easily prepared but gives the poorest results of this class of solutions.

CRESYLECHT VIOLET PROPOSED

For a number of years, we have studied the possibilities of cresylecht violet and about a year ago, made a preliminary report.[1] More recently, however, we have made a number of improvements in our solutions and technic. While the work may be said to be still in an experimental stage, the method given below has yielded results closely approximating those listed above. This I have termed my six-second method. While demonstrating this method

*From Department of Surgical Pathology, St. Anthony's Hospital, Terre Haute, Ind.
Received for publication, July 9, 1924.

to some pathologists recently, they insisted that it should be published and other workers given opportunity to follow up the work in the future.

SOLUTION USED IN THIS METHOD

A number of cresylecht violets and dyes supposed to be cresylecht violet have been submitted to me by the Commission on Standardization of Biological Stains. Of these there is but one which gives the results listed above and this is made by the National Aniline and Chemical Co., Inc.* Some of the foreign dyes show metachromasia but they stain feebly and fail to stain fat, erythrocytes, etc. Their reactions resemble somewhat the polychrome blues.

I make up a concentrated solution of the dye in full strength, white water formalin. This formalin I have secured from the Will Corporation. Other formalins are likely to give poor results because of their high content of methyl alcohol. This solution is filtered and kept tightly corked. It seems to be permanent, and I am using some which was prepared six months ago.

This solution differs in many respects from that described in my former communication. The other solution was dark blue; but this solution appears opaque and almost black in reflected light. It is in fact so opaque that it cannot be used in watch glasses without losing the sections. I have tried lighting my staining tables but only thin films of the stain transmit this light, not enough for practical staining. The color of such thin films is dark blue. It seems that the dye is soluble to greater concentration in this reagent than in water. Moreover the solution is less "greasy" and does not adhere to the glassware as did the one formerly described.

FIXATION

Unfixed tissues may be stained. If time permits, they may be fixed in 10 per cent white water formalin. Alcohols are to be avoided at all costs inasmuch as they dissolve out fat and blood and interfere with freezing methods and staining.

SECTIONING

Frozen sections are used. Thickness of sections will depend upon individual taste. For beautiful preparations the thin section would be preferable; ten to fifteen microns in thickness is better for most tissues in surgical pathology. This thickness will enable the pathologist to study relations "up and down" by changing focus slowly, a point which I have always emphasized but which most men overlook in the attempt to secure thin sections. Moreover when dealing with fresh materials, the sections when thin often roll, wrinkle, ball and cohere, delaying the work. If staining is delayed, the sections may be kept in a vial containing 10 per cent formalin. Many months later, they may be stained by this method.

STAINING

I have stated that the solution is very opaque so that I have done away with watch glasses. The section is spread upon the lifter; excess water is drained

*The solubility of cresylecht violet, as determined in the factory of the National Aniline and Chemical Co., Inc., is as follows at 30° C.

In tap water	0.039 per cent
In distilled water	0.096 per cent
In formalin	1.3 per cent

The formalin was purchased from the Will Corporation.

off but the section is not blotted. The stain is added by means of a pipette. When the section is covered (and one drop will suffice with a small section), approximately six seconds are counted. Then the lifter with section is dipped into a large basin of water* and the section washed free into the water and paddled about gently with the lifter. Lengthening this staining time does not seem to result in overstaining and may be desired in some instances but six seconds will suffice. The solution is corked to avoid evaporation. About one to two seconds will be sufficient for washing section if there is plenty of water.

EXAMINATION

The water-mount may be examined at once. We use the larger slide (2 × 3 in.) and cover by the long rectangle because a number of sections may be glanced at simultaneously if desired.

PERMANENT MOUNTS

The method was designed for diagnostic purposes where haste is imperative and permanent mounts are not satisfactory. I have, however, tried out several methods of mounting, and find that preparations in levulose will be suitable for reference or teaching purposes for a few days. When we find a particular section which we would like to mount, we wash out the cresylecht violet with alcohols and restain with eosin and hematoxylin, though of course we sacrifice many details in so doing. Although designed only for rapid diagnostic work, many pathologists have insisted that I also devise some method for permanent mounts that these preparations may be used for teaching. However, thus far I am compelled to admit failure.

RÉSUMÉ

In our hands, the method has given some beautiful pictures and is suggested for further study by pathologists. It may be possible to improve upon this solution, but even in its present status, it is so superior to the polychrome methylene blue methods that they may well be abandoned and attention centered on cresylecht violet.

At first thought it might seem that we are asking too much of an oxazin in the matter of the metachromasia listed above: I do not think so. I have always felt that such reactions could be secured with sufficient effort and study, and this has been the reason for my investigations. It might seem farfetched practicability to stain fat with other elements, and so it seemed to me when I discovered this property of cresylecht violet in 1921. But I have learned that it often explains "spaces" overlooked in some preparations. Thus in some mixed connective tissue tumors, this has been proved. Quite recently I noted what seemed to be a fat metaplasia in a cervix uteri, and which would not have been revealed by other methods. It also serves to show diffuse metastases in adipose tissue of omentum, etc., which might otherwise be overlooked.

*A large, shallow dish is suitable. I prefer a Pyrex cake dish or baking dish about 8 x 8 in.

The same may be said in regard to the pea-green blood cells and the metachromasia of muscle versus nuclei in myopathic lesions of appendix. The same is true in some granulations in the differentiation of fibrils and fibrin.

REFERENCE

[1]Williams, B. G. R.: Cresylecht Violet, A Rare Dye, JOUR. LAB. AND CLIN. MED., viii, No. 4.

THE RED CORPUSCLE SUSPENSION FOR THE KOLMER-WASSERMANN REACTION*

BY C. E. REYNER, B.S., DETROIT, MICHIGAN

IN the standardized Wassermann technic, proposed by Kolmer[1, 2] and used in this laboratory for two years, it has been found that the 2 per cent red corpuscle suspension of sheep's blood at times hemolyzes while standing overnight in the ice box. We use sheep's blood, secured from a slaughter house, in a solution containing 1.5 per cent sodium citrate and 0.85 per cent sodium chloride and it is frequently three or four days old when used. When hemolysis occurs in the red cell suspension, while standing overnight in the ice box, the positive results are not satisfactory to read and are weaker on account of the apparently increased fragility of the cells. This difficulty has been entirely overcome by means of the well-known fact that red cells hemolyze more rapidly when in dilute suspension than when concentrated. The method finally adopted follows:

The cells are washed with physiologic saline in the manner described by Kolmer and Brown.[3] Centrifuge the citrated blood without dilution, remove the supernatant fluid and add 3 to 5 volumes physiologic saline and centrifuge again; remove the supernatant fluid and add 3 to 5 volumes of the saline, centrifuge and remove the supernatant fluid. For measurement, the washed cells are packed into 15 c.c. graduated centrifuge tubes by centrifuging for five

TABLE I

Days old	1	2	3	4	5	6	7	8
2% Suspens.	N.H.	N.H.	T.H.	P.H.	M.H.	* *	* *	* *
Packed cells	N.H.	N.H.	N.H.	N.H.	N.H.	N.H.	P.H.	M.H.
Formal. 2% Susp.	N.H.	N.H.	N.H.	N.H.	N.H.	N.H.	N.H.	P.H.
Packed cells Form.	N.H.	N.H.	N.H.	N.H.	N.H.	N.H.	N.H.	T.H.

N.H.—No Hemolysis. T.H.—Trace of Hemolysis. P.H.—Partial Hemolysis. M.H.—Marked Hemolysis.

TABLE II

Days old	1	2	3	4	5	6	7	8
No Preserv.	B.R.	B.R.	B.R.	B.R.	D.R.	P.	M.H.	M.H.
Formalized	B.R.	B.R.	B.R.	B.R.	B.R.	B.R.	B.R.	B.R.

B.R.—Bright Red. D.R.—Dark Red. P.—Purple. M.H.—Marked Hemolysis.

*From the Laboratories of the Henry Ford Hospital, Detroit, Michigan.
Received for publication, July 9, 1924.

TABLE III

Days old	1		2		3		4		5		6		7		8	
	I.H.	C.H.	I.H.	C.H.	I.H.	C.H.	I.H.	C.H.	I.H.	C.H.	I.H.	C.H.	I.H.	C.H.	I.H.	C.H.
No preserv.	.800	.520	.800	.520	.800	.520	.800	.520	.800	.560	.800	.560	.800	.560	.840	.560
Formalized	.760	.520	.760	.520	.760	.520	.760	.520	.800	.520	.800	.520	.800	.520	.840	.520

I.H.—Initial Hemolysis. C.H.—Complete Hemolysis.

minutes at 2,000 R.P.M. The tops of the cell columns are marked with a wax pencil and all tubes of packed cells are put away in the ice box except one which is used to prepare the cell suspension for the titrations. In the morning, when the series of "set ups" is ready for the final incubation, the packed cells are simply diluted with physiologic saline to make a 2 per cent suspension. The cells, kept in this way in the ice box overnight, have been found to hemolyze very little, if any, even when the cells are several days old.

When the dilute 2 per cent suspension of cells was kept overnight hemolysis was observed when the blood was three days old, whereas, when the packed cells were kept in the ice box overnight and then diluted to 2 per cent with 0.85 per cent saline, no hemolysis was observed on the sixth day. (See Table I.)

The apparently increased fragility of the cells kept overnight in a 2 per cent suspension is not borne out by fragility tests. For four days the initial and complete hemolytic points of the red cells remained constant at 0.800 per cent and 0.520 per cent sodium chloride respectively. It retained the levels of .800/.560 for three days and, on the eighth day, it was .840/560. (See Table III.)

When a 2 per cent cell suspension, prepared from sheep's blood six days old and permitted to stand in the ice box overnight, is compared with a suspension prepared from packed cells kept overnight in the ice box, a marked difference is observed.

THE USE OF FORMALIN AS A PRESERVATIVE OF RED CORPUSCLES

Kolmer and Brown[3] suggest the use of formalin as a preservative of red corpuscles. It is shown that a 2 per cent suspension of formalized sheep's corpuscles did not hemolyze while standing overnight in the ice box until the cells were over eight days old. Keeping packed formalized cells overnight did not show hemolysis until the cells were eight days old and then only a trace of hemolysis was observed. (See Table I.)

A comparison of the gross appearance of plain and formalized sheep's blood, as shown by Table II, reveals the fact that plain blood remains a bright red color for four days and then begins to get dark until, on the sixth day, it has a purple color and is totally unfit for use, and the formalized blood retaines its original bright red color for the entire period of eight days. The hardening of the blood cells with formalin has a slight effect on the fragility of the cells, as indicated by Table III, in that it decreases their fragility.

Difficulty, due to the hemolyzing of cells kept overnight in

the ice box while in dilute suspension, may be avoided by keeping the packed cells overnight and diluting just before use.

Formalizing sheep's cells enables one to keep them for a week or longer. It has been found that even formalized sheep's cells are more satisfactory if kept overnight packed, instead of in a 2 per cent suspension.

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ON THE NONPROTEIN SULPHUR OF THE BLOOD*

By Max Kahn, M.A., M.D., Ph.D., and Robert S. Postmontier, B.S.,
New York

FOR the past several years,† an attempt was made in our laboratory to study the nonprotein sulphur fractions of the blood. We have finally adopted the following methods:

Total Nonprotein Sulphur of Blood.—Ten c.c. of oxalated blood collected in the usual way from an arm vein, are treated with 90 c.c. of 2½ per cent trichloracetic acid to precipitate the total blood proteins, according to the method of Greenwald. After letting stand for half an hour, 80 c.c. of the filtrate are evaporated over a hot water-bath to half its volume, 10 c.c. of Benedict's sulphur solution are added, and the sulphur oxidized to sulphate, which is then determined gravimetrically as barium sulphate, according to Benedict's method.

Total Sulphate Sulphur of the Blood.—The method of Vansteenberghe and Bauzil was used. The reagents necessary are: Barium chloride, 12.2 gm. crystallized salt per liter; N/10 hydrochloric acid; 15 per cent sodium carbonate solution; 0.1 per cent aqueous solution of methyl orange; 10 per cent ammonium chloride solution; 36 per cent acetic acid solution. Ten c.c. of oxalated blood are treated with 40 c.c. of the ammonium chloride solution, and 1 c.c. of acetic acid added. The liquid is heated to boiling with stirring to coagulate the albumin. The liquid is then filtered, the filter washed once or twice, and the filtrate evaporated down to 10 c.c. It is then acidified with hydrochloric acid, and exactly 5 c.c. of the barium chloride solution added. The liquid is boiled for one minute, centrifuged, or filtered through talc, and an excess of the sodium carbonate solution added to the filtrate. The whole is then centrifuged or filtered, and the precipitate of barium carbonate washed

*From the the Dept. of Laboratories, Beth Israel Hospital, New York City.
Received for publication, March 16, 1922.

†M. Kahn: Proceedings of the Society for Experimental Biology and Medicine, 1919, xvi, 138-139.

until the washings are neutral to litmus. The precipitate is then washed into a beaker, 10 drops of methyl orange solution added, and titration made with the N/10 hydrochloric acid. The end-point must match the tint obtained when adding ten drops of indicator to 0.5 c.c. N/1 hydrochloric acid in a volume of water equal to that used in the experiment.

The results that we have obtained by this method are extremely interesting from the clinical point of view. The figures should be considered as approximate rather than exact, and we would rather have the method considered as clinical-chemical than as purely chemical.

CARCINOMA

	Total Sulphur as SO_4	Inorganic and Etheral Sulphur as SO_4
1. Stomach	8.1	6.4
2. Stomach	10.2	8.5
3. Uterus	9.4	7.7
4. Uterus	9.5	7.2
5. Breast	7.5	5.2
6. Breast	5.6	4.1
7. Esophagus	11.0	8.3

SKIN DISEASES

	Total Sulphur as SO_4	Inorganic Sulphur as SO_4
1. Acne	3.7	2.1
2. "	2.5	1.9
3. "	2.8	2.2
4. Eczema	4.2	3.5
5. "	2.9	1.9
6. "	2.5	1.8
7. Psoriasis	7.3	6.7
8. "	7.4	6.2
9. "	6.4	5.4
10. "	8.2	7.2
11. "	5.6	4.2

CHRONIC INFECTIONS

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
1. Tuberculosis	7.2	5.9
2. "	7.5	6.2
3. "	8.9	7.1
4. "	11.2	9.3
5. "	4.7	3.2
6. Syphilis	4.8	3.1
7. "	9.2	8.1
8. "	8.5	7.4
9. "	8.6	7.2
10. "	7.7	6.4

ACUTE INFECTIONS

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
1. Typhoid	3.2	2.2
2. "	1.9	1.4
3. "	1.7	1.3
4. "	2.8	1.9
5. "	3.5	2.8
6. "	3.4	3.1
7. Pneumonia	3.7	2.8

ACUTE INFECTIONS—CONT'D

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
8. Pneumonia	5.8	4.2
9. "	8.6	7.5
10. "	8.4	7.8
11. "	3.2	2.6
12. "	2.9	2.3
13. "	4.3	3.5
14. Typhus	4.4	3.7
15. Septicemia	3.8	2.9
16. "	2.9	2.4

METABOLIC DISEASES

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
1. Diabetes	7.5	6.6
2. "	12.3	9.7
3. "	4.2	3.7
4. "	5.7	4.2
5. "	5.9	5.1
6. "	6.8	6.1
7. "	7.4	6.6
8. "	8.7	7.2
9. "	4.9	3.7
10. "	4.5	3.5
11. Gout	4.3	3.8
12. Basedow	7.2	6.5
13. "	7.5	6.2

BLOOD DISEASES

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
1. Secondary Anemia	3.7	3.1
2. " "	2.9	2.2
3. " "	2.8	2.1
4. Pernicious Anemia	7.5	6.6
5. " "	8.7	7.8
6. Chlorosis	2.2	1.8
7. Acute Leucemia	8.5	7.7
8. Hodgkin's Disease	2.9	2.2
9. " "	3.1	2.7
10. Myelogenous Leucemia	5.6	5.1
11. " "	5.7	5.0
12. " "	6.2	5.5
13. Lymphatic Leucemia	3.9	3.1
14. " "	3.8	2.9
15. " "	2.6	1.9
16. Von Jaksch Anemia	2.2	1.7
17. Hemorrhage	2.7	1.5

RENAL DISEASES

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
1. Acute Nephritis	14.3	12.1
2. " "	11.2	9.3
3. " "	15.7	11.2
4. Chronic Parench. Nephritis	10.3	8.4
5. " " "	2.5	1.8
6. " " "	7.8	6.2
7. " " "	6.2	5.9
8. Chr. Interstitial Nephritis	2.7	1.9
9. " " "	2.9	1.8
10. " " "	8.5	7.3
11. " " "	8.6	7.4

RENAL DISEASES—CONT'D

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
12. Chr. Interstitial Nephritis	10.7	8.9
13. " " "	7.2	6.2
14. " " "	5.3	4.1
15. Congestion of Cardiac Dis.	4.5	3.7
16. " " " "	2.8	2.2
17. Uremia	12.9	10.5

SURGICAL CONDITIONS

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
1. Appendicitis	2.7	2.1
2. "	1.8	1.2
3. "	2.9	2.2
4. "	2.5	1.9
5. Fracture	3.2	2.6
6. "	2.8	2.1
7. "	3.5	2.9
8. "	3.6	3.0
9. Hernia	3.4	2.8
10. "	3.7	3.1
11. "	3.8	2.95
12. "	2.5	1.9
13. "	3.3	2.7
14. Pregnancy	4.1	3.6
15. "	4.5	3.5
16. Postpartum	2.9	2.1
17. "	3.2	2.4

It will be seen from the results shown in the various tables that distinct changes in the nonprotein sulphur content of the blood are present in certain conditions of disease, as for example carcinoma, psoriasis, certain chronic infections, disturbances of the renal apparatus, and advanced diabetes. It has been observed by several workers in this field that there are distinct disturbances in the sulphur metabolism in carcinoma;* no data of disturbance in this fraction of metabolic exchange has been recorded in the other conditions enumerated. In psoriasis this may be an index of diagnosis and therapy. In nephric disease the increase is due to a retention similar to the retention of the nitrogen fractions.

In normal conditions of body metabolism the figures that we have obtained are 2 to 4 mg. (per 100 c.c. of blood) of total sulphur and 1 to 3 mg. of total sulphate sulphur. In states of "dysbolism"† these figures are markedly increased as the tables show. We are continuing our work along this line.

*M. Kahn: Sulphur Metabolism in Carcinoma, *The Journal of Cancer Research*, 1917, ii, 379-391.

†I suggest this word as a substitute for the expression "disturbed states of metabolism," which is occasionally rather an unwieldly phrase.

CERTIFIED STAINS—WHAT THEY ARE, AND HOW TO OBTAIN THEM

By H. J. Conn,* Geneva, N. Y.

IT may not be generally realized that in the case of some of the most important biologic stains it is possible to make a purchase knowing that a sample of the identical batch from which the purchase is made has been tested by laboratory workers skilled in the procedures for which that stain is ordinarily used. The stains in question are those which have been approved by the Commission on Standardization of Biological Stains.

This Commission was organized by the National Research Council, and is now in close affiliation with it, although no longer a part of the larger organization. It has been put on an independent footing and receives its support from various sources: first, an appropriation made from time to time by the Chemical Foundation; second, certain contributions made by a considerable number of the leading dealers in general biologic supplies; and third, small sums received from stain manufacturers in return for the services of the Commission in testing their products and for certification labels supplied them for use on batches of stain found to be satisfactory. The last source of income is never likely to be large, because the total volume of business in stains is too small to bear an expense of this kind without making the prices of the products prohibitive. To the donors of the contributions composing the other two sources of income, the Commission wishes to express its sincere gratitude and to assure them that it appreciates the entirely unselfish spirit in which the contributions are made. To avoid complications the Commission has adopted the policy of not accepting gifts from any concern having stains of its own which it wishes to have approved and of whom a selfish motive might be suspected.

The plan adopted by the Commission for standardizing stains is to approve definite batches submitted to it and found satisfactory. The reason for this policy is the great difficulty of securing two batches of any stain which are exactly alike, so that it is not safe to recommend the entire product of any concern. Biologic stains are still too little understood to make it possible to draw up definite chemical standards which will assure a satisfactory product.

When a batch has been found satisfactory it is sold with a special label on it furnished by the Commission bearing this statement: "Found satisfactory by Commission on Standardization of Biological Stains for { above / purposes mentioned purposes. Use for other purposes not contraindicated unmentioned on main label.

*Chairman of Commission on Standardization of Biological Stains.

Received for publication July 7, 1924.

less specifically so stated { above. / on main label. }" Two alternate forms of wording are used, as just indicated, because two forms of label are furnished by the Commission, one of which becomes the main label on the package, while the other is to be used in addition to the main label.

Certain stains have been put on the market labeled "Certified," but without the Commission label. Such labeling is of no value to the purchaser, as it merely indicates certification by the manufacturer himself.

Certification of stains by this batch method has to proceed slowly as specifications have to be worked out for each stain considered; but the following stains have already been put on the certification basis: Methylene blue, basic fuchsin, acid fuchsin, hematoxylin, safranin O, and pyronin. The companies to whom certification for some or all of these stains has been issued are: Empire Biochemical Co., Hartman-Leddon Co.; MacAndrews and Forbes, National Anilin and Chemical Co. and Providence Chemical Laboratories. Some of these companies do not carry all the previously mentioned certified products; but the majority of the dealers in general laboratory supplies plan to carry the certified stains as fast as certification is issued. The dealers known at present to be cooperating in this way are: Braun Knecht Heimann Co., Central Scientific Co., Denver Fire Clay Co., E. F. Mahady Co., E. H. Sargent and Co., Scientific Materials Co., Arthur H. Thomas Co., and the Will Corporation. Others may also carry these stains; and if so the Commission will be glad to have its attention called to their omission.

The chairman of the Commission is always glad to answer questions concerning stains. Considerable information has accumulated as the result of the investigations already carried on, and often a specific question can be answered so as to show a prospective purchaser just where to go for the particular stain he wishes. The chairman's address is: Agricultural Experiment Station, Geneva, N. Y.

TRANSACTIONS

THIRD ANNUAL CONVENTION AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS, ROCHESTER, MINNESOTA

June 5-7, 1924

(Continued)

DR. FREDERICK E. SONDERN, New York, Chairman, gave a **Provisional Report of Committee on Laboratories.**

In the course of the winter, your President appointed a committee, the object of which was to consider the inefficient private laboratory. The members of this committee live in all parts of the United States, and it was difficult, or really impossible, to get a meeting of this committee. Those that I could get together did meet in New York, so this, if we may call it a report, is one framed by a relatively small number of the committee. There are in this report certain recommendations. They may be considered rather radical by many; and in order that the members of the Association might have an opportunity to think them over and talk them over with one another, we requested our President to let me present this matter now, and then we can take it up at the regular business meeting on Saturday to do with it as you think best.

It is an established fact not only in this country, but also overseas, that a large amount of clinical laboratory work is being done by persons absolutely unqualified by training and experience. This state of affairs has during recent years engaged increasing attention particularly of those interested in public health, and a remedy is eagerly sought.

Practically speaking, clinical laboratory work is the result of a demand of the physician for aid in diagnosis, and were physicians as a class, exacting in the laboratory service rendered, the existing evil would soon come to an end.

These constantly increasing justified complaints relative to inefficient and misleading laboratory service, particularly in our larger cities, must have the serious attention of such bodies as our Association, and a remedy should be found to make very probable legislative control unnecessary or to guide such control if in public opinion it should become necessary.

Specific happenings in this connection may tend to emphasize the brief statements which have been made. Some eight years ago or more, the New York City Commissioner of Health, Haven Emerson, called attention to the large number of complaints of incompetent work done by many private laboratories in New York City, and expressed the hope that the New York Academy of Medicine might do something to remedy the condition. In consequence, the Public Health Committee of the Academy made an investigation, and, as a result advised that the Board of Health frame rules and regulations under which private laboratories should be allowed to operate. Beyond the requirements of a license, nothing has been done to date.

About the same time, a similar complaint was made by officers of the American Medical Association to some of the private clinical pathologists of Chicago, with an unofficial request that the laboratory men themselves establish an organization for laboratory standardization and control, which, if suitable and efficient, should win the support of the American Medical Association. An earnest effort in this direction was made under the leadership of Hektoen and others, but the World War prevented the success of this undertaking. It was also about this time that a study of this problem was made by the New York Bureau of Municipal Research, and in an exhaustive report published by this body, the authority of the City Department of Health in the matter was demonstrated under

existing law and compulsory control was strongly advocated in the interest of public health. Hospital surveys under the auspices of the American College of Surgeons, and the activities of the Council of Medical Education and Hospitals of the American Medical Association have given increasing attention to laboratory personnel and equipment.

As it was impossible for all the members of your Committee to meet to consider the problems under discussion, some of the written comments of the absentees may be of interest here.

"Pathologists are often connected with hospitals or private laboratories performing no other function than to give a false sense of responsibility to these laboratories."

"It is most desirable that medical laboratories have physicians who have mastered their specialty in direct charge of the work."

Enough has been said to prove that the danger of existing conditions is generally recognized and therefore it scarcely seems necessary to review in detail evidence of its actual existence. There is no one active in clinical pathology who cannot detail a number of laboratories conducted by insufficiently trained technicians, and even by medical graduates who have no knowledge of laboratory technic, and employ incompetent technicians to do the actual laboratory work. It is also not uncommon to find hospital laboratories equally inefficient for similar reasons, to say nothing of the instances when a competent clinical pathologist through stress of private work exerts no control over his inefficient institution laboratory.

Admitting existing conditions, what can our Association do to remedy these evils, and what will happen if the issue is avoided? These are important questions with professional and economic significance which confront the qualified physician who specializes in clinical pathology and it would seem urgent for our Association to assume as one of its legitimate functions the duty of at least attempting a solution. A careful survey of the situation is urged and a critical examination of plans for relief should become a duty. While self-appointed censorship is on the whole an undesirable method, still if no other scheme is available, it may be necessary to come to it.

For the purpose of opening a discussion which may lead to constructive action by the Association, it is suggested that a Commission be appointed to formulate a method by which the laboratories of Members of the Association may on request secure what may be known as a Certificate of Approval of the American Association of Clinical Pathologists. This method might include qualifications of personnel, standardization of laboratory procedure possibly controlled by inspection, periodic report on submitted specimens or by such method as the Commission may think wise. The details of such requirements and standards might be relatively simple at first, to be improved and perfected as the plan develops and its need and desirability become more evident. At all events, this official approval should be of such high standard as to secure at sight the unqualified endorsement of such bodies as the American College of Surgeons, the American Medical Association and similar organizations.

This procedure, to be sure, will not assure the elimination of the inefficient laboratory because it will not succeed in making the careless or incompetent physician or the lay public pay proper attention to securing efficient laboratory service. It will, however, convince the thinking medical men of the country and the leading organizations of which they are members, that a laboratory standard has been established which they cannot ignore.

We live in an era of profuse legislative activity in the control of almost every human endeavor, and if this should extend to a consideration of laboratories, aside from such activity as a purely medical professional function, as it well may, the existence of an established standard endorsed by leading medical organizations, might easily shape a proper course of action. In the absence of such organization, however, such effort might easily grow to bizarre proportions, of which we have so many examples in other fields at the present time.

The only existing practical standardization and control of public health laboratories is that of the New York State Department of Health, and mention of it has purposely been delayed until now as it may best serve here by way of an example of what can be

done. This plan was conceived and elaborated by A. B. Wadsworth, Director of the Laboratories of the New York State Department of Health, and carried out by and under the supervision of Ruth Gilbert, head of the Diagnostic Laboratory of the Department.

The plan consists of the annual approval of any public health laboratory in the State for specific diagnostic laboratory procedures if efficient personnel, suitable equipment and agreements relative to methods used meet with the standard established by the Department. These requirements are met by details of personnel and equipment stated on the application blank, a series of agreements relative to methods signed by the applicant, inspection by a representative of the Department, and the occasional submission of specimens for examination which are reported to the Department. Samples of the blanks, agreements and certificates are submitted herewith.

This system is of decided value to the Department in estimating the reliability of the laboratories throughout the State and their value in safeguarding public health. It is in the main characterized by courteous cooperation and constructive criticism in which way it renders a valuable service to the laboratory itself, and its existence, without any doubt, urges higher laboratory standards.

A similar system of voluntary submission to laboratory control exerted by this Association might be the means of convincing the thinking members of the medical profession that an absolute laboratory standard has been established. It will be of little or no use unless a very large proportion of the truly efficient laboratories of the country join in the movement, and they will not do so unless the control is exercised by most competent authority in a tactful, courteous and constructive manner.

The object of this report is to lay the matter before you for discussion, and if you see virtue in it as we do, to recommend the appointment of a commission to work out the many essential details and to present to you a complete working plan.

DISCUSSION

President MacCarty.—I sincerely hope we have all paid close attention to this report. I know how seriously the Committee has taken this problem, and I know that it has a very constructive, charitable and tolerant plan. The members of the committee mean to carry out the reform they desire by education rather than by legislation, and I think that that was quite evident in Sondern's report. I hope you will all think about this very seriously, and discuss it before our business meeting on Saturday, because I think we should do something with this very excellent report.

DR. L. A. TURLEY, Oklahoma City, Okla., read a paper entitled **Spinous Cell Carcinoma.** (For original article, see page 272.)

DISCUSSION

Dr. Turley.—I want to say, personally, that I have experienced some disappointment in the discussion of the papers up to this time, and I hope there will be some discussion of my paper. This morning I heard a man say, "I would have discussed a certain paper yesterday, but I did not agree with the author." I hope no one will hesitate to discuss this paper, because of the fact that he does not agree with me. I can always learn something from a man who disagrees with me, and that is one of my purposes in life—to learn as much as I can. (Paper read)

Dr. Hillkowitz.—I do not think the appeal of Turley for a discussion of his paper should be left unheeded, if for no other reason than disagreeing with him, which I am constrained to do, particularly in view of the fact that our President this morning told us that there are 1138 different pathologic entities in our nomenclature, and today one more I think would be like the proverbial straw that would break our back. Turley's nomenclature would be acceptable if it would add anything from the standpoint of helping the pathologist, either in diagnosis or prognosis. As MacCarty pointed out, the average clinician wants to know whether the tumor is benign or malignant, or how benign or malignant it is. Now it seems like splitting hairs merely to state that the squamous cell is not, strictly speak-

ing, a squamous cell, but has its various dimensions and three points in space. So far as the prognosis of the patient is concerned, we are aware that the squamous cell variety is more apt to produce metastasis, but to inject a new classification of spinous cell and say categorically that certain tumors arise from the spinous cells, thus overthrowing, if I understand it rightly, our previous conception that there is a transition from the lower layers, gradually growing into the spinous cells, and then into the dead cells at the top; such a classification is a revolutionary step which I am not yet willing to accept. Nevertheless, you will pardon me, if I express the usual compliments of gratitude to Dr. Turley for bringing this information to our attention. It is a subject well worthy of discussion and consideration.

Dr. Turley (in closing).—I am very glad that the Doctor's discussion has brought these points out. So far as the new term is concerned, and MacCarty's statement that there are now listed 1138 different kinds of tumors, I heartily agree with both men that we should simplify the nomenclature and reduce the number of tumors if possible. I recently received a letter from an eminent pathologist and clinician in this country, who has a man working under him, who has made out, I think, something like twelve different kinds of tumors that are, all of them, when you come to examine them, this same spinous cell carcinoma. This I should say was superfluous addition of names and terms. I think we should have a simple classification and that we should stick to it; but I think the point is also true, if the pathologists are going to talk about the same thing, that we should have the same terms so that we understand exactly what they mean. For instance, when a patient comes from one part of the country to another with a diagnosis of a certain type of carcinoma, not only the one who made the diagnosis but the one who reviews the case will know just what the diagnosis means. Let us say he has an epidermoid carcinoma, which as the Doctor pointed out, has a certain type of malignancy; now if that includes a whole variety of tumors, for example, all possible tumors that might develop from stratified squamous epithelium the diagnosis means as little as the term "bilious." It is as misleading to the surgeon and clinician to restrict our number of kinds, if they are essentially different, as it is to have too many kinds, because different varieties of tumors have different habits of malignancy and growth. A definition should be inclusive and exclusive; that is, one should include all of a certain variety and exclude all the other varieties. In referring to the second point that Hillkowitz brought out, I would like to ask him if we should keep all old theories simply because they are old, or because it is going to disturb our equanimity by requiring us to learn something new? If it can be shown that the old theory is erroneous, why not change to a correct one?

Dr. E. H. Ruediger, Los Angeles, California, read a paper entitled **Quantitative Wassermann Test.** (For original article, see page 303.)

DISCUSSION

Dr. John A. Kolmer.—I thoroughly agree with the principle of using a quantitative Wassermann reaction, because of a very important biologic reason. If one conducts the test with a constant or fixed dose of serum, as one-tenth or two-tenths of a c.c., we may occasionally observe that with these amounts of serum that the reaction is weak, or, indeed, might be negative with the serum of a syphilitic. With my own method, I think this happens about once or twice in each one hundred sera, so that from the standpoint of accuracy there is probably this very important reason for using the serum in at least two or three doses. I am inclined to think that the phenomenon can be observed with practically any method in common use at the present time, irrespective of the technic being employed; I believe it is well for those who are using one dose of serum with their present method to modify it slightly in order to carry one or two extra tubes with a smaller amount of serum. We have not noticed this so far with spinal fluids and I do not know why the phenomenon occurs. Naturally, of course, one first thinks of the presence of antisheep hemolysin. Our own experience, however, shows that while this might be one factor, it is not the whole story. It might be, as I think Sanford has suggested, a phenomenon due

to the presence of salts in the plasma. We do not know just the mechanism concerned, but I think it constitutes a very important biologic reason for conducting our test, irrespective of the method we are employing, with at least two or three doses of the serum, in order to obtain a greater degree of accuracy insofar as positive and negative is concerned. Another, much less important reason, is to give a graphic record of serologic improvement in the treatment of syphilis. I think this is quite important, but much less important than the first reason; but often, physicians who are engaged in the treatment of syphilis, after noting considerable clinical improvement in the patient, are rather surprised that the serologic reaction is without improvement. With the old method, the reactions continue to be plus-4, and if the physician acquaints his patient with this result, it can be very depressing indeed. As a matter of fact, I think those of us here who are engaged in the treatment of syphilis have learned not to acquaint our patients always with the results of the Wassermann test, because it is frequently very depressing. However, when a quantitative reaction is conducted, we can usually show by using varying amounts of serum, a greater serologic improvement, and this is quite important to the physician and his patient. This constitutes the important reason for conducting a quantitative method, irrespective of the method that any of us may be using.

Dr. A. H. Sanford.—I heartily second everything Kolmer has said. About two years ago, it seemed to us rather formidable to attempt to do quantitative Wassermanns, doing as many as we do every day, but since 1922 we have done quantitative Wassermanns on spinal fluids, and on serum, when it is requested, to the full five-tube quantity, and in two-tube quantities in all the tests. We are far more satisfied ourselves and we know that the dermatologist is far more satisfied.

Dr. Marquez.—My experience with the Wassermann reaction is rather unsatisfactory. Whether that is due to my technic, or due to the imperfect clinical reports that we receive in the laboratory, I do not know. I have followed Kolmer's technic to determine the Wassermann reaction, and I have come in contact with clinicians who criticize his reaction, for the simple reason that in two instances when my curve came down toward the negative line in blood sera, the patient was developing symptoms of tabes, and they could not understand why this was so. We laboratory men can readily appreciate that; but our fight is with the practicing physicians. If we could only convince them that this quantitative reaction of Wassermann is of value, in spite of its occasional failures, we would accomplish very much toward the standardization of our quantitative Wassermann test. It is to be admitted that in time we shall have the standardized Wassermann reaction, and the physicians will appreciate and read it with just as much understanding as we do, so we must not get impatient with our brother practicing clinicians.

Dr. Ruediger.—This phenomenon of a negative result with human serum of low dilution and a positive result with the human serum in a higher dilution is not entirely due to the normal antisheep hemolytic amboceptor in the human serum, because it is also seen when the antihuman hemolytic system is used in which case there is no such excess of hemolytic amboceptor. I cannot explain the cause; about four years ago, in a report on this subject, I showed that it completely disappeared with aging of the serum. I preserved the human serum, tested it from time to time and found that it usually disappeared in less than a month. So far as the attitude of the clinician toward the quantitative Wassermann test is concerned, we sometimes meet opposition from those unfamiliar with it. Those who are not used to quantitative reports can usually be persuaded to accept them; but after they have become used to quantitative reports they usually cannot be persuaded to go back to the qualitative reports.

The Journal of Laboratory and Clinical Medicine

VOL. X ST. LOUIS, MO., JANUARY, 1925 NO. 4

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EDITORIALS

Carbon Dioxid as a Respiratory Stimulant in Gas Poisoning

THREE years ago Henderson, Haggard and Scott first recommended the use of small concentrations of carbon dioxid with oxygen in the treatment of carbon monoxid poisoning. Since then the method has demonstrated its value and is being utilized in various parts of the country.

Carbon monoxid appears to exert no action within the body other than its combination with hemoglobin, thereby diminishing the oxygen transporting capacity of the blood. One who has inhaled sufficient carbon monoxid to produce toxic symptoms suffers primarily from tissue anoxemia, and especially from cerebral anoxemia. The carbon monoxid-hemoglobin combination, while firmer than the usual oxygen-hemoglobin combination, is notwithstanding a reversable reaction and after removal from contact with carbon monoxid, the victim will, provided he is still breathing, rid himself of the absorbed gas within a relatively short interval.

Oxygen alone has in the past been rather ineffectual in the treatment of gas poisoning. Several factors contribute to this. There is no efficient apparatus generally available, and treatment is usually too long delayed to be effective. If the patient has been removed from the poisonous atmosphere, and provided he continues to breathe, all of the carbon monoxid will have passed off through the expired air in four or five hours. Oxygen will have taken its place. After this the administration of oxygen becomes superfluous. If symptoms persist they are due not to continued depreciation of oxygen, but to tissue damage already accomplished by the pre-existing oxygen want.

Individuals poisoned for some hours suffer so great depression of the respiratory center that even though oxygen be administered, the breathing is too shallow for good results. The administration of carbon dioxid to stimulate the respiratory center has been used for some time in conjunction with ether anesthesia, and Henderson and Haggard have applied this principle to the treatment of gas poisoning.

Experimentally, gassed animals were given oxygen containing 10 per cent of carbon dioxid. Within twenty minutes practically all of the carbon monoxid had been passed from the lungs. Those animals treated with air plus carbon dioxid, eliminated the poisonous gas in eighty minutes. The controls required as long as one hundred and forty minutes for the removal. They found carbon dioxid in 10 per cent concentration to be so effective a stimulant of the respiratory center that the breathing became far more rapid than normal, a condition of true dyspnea developing. Only one disadvantage in the method, observed in the earlier animal experiments where large amounts of carbon dioxid were used, consisted in a deleterious action on the heart, which was overcome by the administration of atropin.

In experiments on themselves, however, the authors found that 5 per cent concentration of carbon dioxid in oxygen was sufficient to produce the desired effect and this concentration had no deleterious action upon the heart. Their observations were applied in practice to several cases of gassing in New York City with thoroughly satisfactory results. Even those victims so seriously gassed as to require artificial respiration, once they had inhaled sufficient carbon dioxid to stimulate the respiratory center, exhibited rapid increase in the rate and depth of breathing until they were inhaling from twenty-five to thirty liters of the gas mixture per minute. The carbon monoxid in the blood was reduced with extreme rapidity, the concentration falling from asphyxial amounts to practically negligible quantities within from fifteen to twenty minutes.

Not only were the immediate results most favorable but the incidence of "asphyxial complications," particularly bronchopneumonia, was correspondingly reduced.

As is often the case, successful empirical treatment antedates rational therapy by many years. Four cases of accidental carbon monoxid asphyxia in Richmond, Va., were recently treated by the Henderson-Haggard method with excellent results. Discussion of the event brought forth the information that twenty years ago an employee in the Richmond Gas Plant had discovered quite by accident that beer was the best antidote in gas poisoning. Un-

til the advent of prohibition it had been a tradition among the employees to get as much beer as possible into the stomach when one of their fellow workers had been gassed. During recent years ginger ale has been substituted. Carbon dioxid appears to be absorbed from the stomach. It is absorbed with quite surprising rapidity from the peritoneal cavity.

Patients treated by the Henderson-Haggard method inhale roughly one liter of carbon dioxid at atmospheric pressure per minute. It is of course uncertain whether the volume of carbon dioxid absorbed from beer or ginger ale in the amounts that can be retained by the stomach, would be sufficient for pronounced stimulative effect upon the respiratory center. But there can be no gainsaying the fact that the administration of beer and ginger ale appeared to be beneficial in a sufficient number of cases, probably chiefly mild and early ones, so that this emergency relief measure had become traditional among the employees of the gas plant.

—*W. T. V.*

The Hospital Laboratory and Research

IF one may judge by hospital reports, the activity and efficiency of the hospital laboratory is most frequently indicated by the number of examinations made during the year; but a far more inclusive and reliable criterion, affording a more accurate estimation of the true efficiency of the laboratory, as well as indicating in a decisive manner its true place in the hospital's activities, is to note the degree to which it initiates, seizes upon, and develops opportunities for laboratory and clinical research and investigation. The index to these activities is best indicated by the publications issued from the laboratory during the period covered by the hospital report.

It would seem that there is a tendency, especially in the smaller hospitals, to look upon research as connoting, far-reaching problems of vast magnitude possible only to institutions especially equipped for the purpose. This thought, however, is fallacious and leads, too often, to lethargy, inertia, and neglect of the opportunities and material at hand and clamoring for recognition.

There is no greater problem nor any of more import than the study of disease and the means available for its recognition and treatment. New methods of laboratory investigation and modifications of old ones are continually being advanced. These must be evaluated by comparative trial and their significance determined.

The study of disease and the development of its mechanism and manifestations is the study and recording of minutia. There is no hospital the thoughtful consideration of whose laboratory records, if carefully kept and representing careful and consistently thorough work, cannot be made profitable—if not to medicine as a whole, then to the personnel of the hospital itself. There are few, indeed, in which some studies cannot be carried on and which cannot—as they should—contribute their mite to medical knowledge and progress.

Even in those where the work, perhaps because of lack of enthusiasm or initiative on the part of the director, or lack of cooperation on the part of the clinical staff, has assumed a routine, hack character, if records are kept and studied something must come of it; either such and such a routine examination is of no real value and should be discarded, or it is informative or could be made so—in either case an addition has been made to the sum of existing information.

Every hospital with a maternity and, especially, an obstetrical dispensary, however small, is a potential source of information as to the significance of the various blood-chemistry findings in pregnancy and its complications; the medical wards furnish unlimited opportunities for the accumulation of similar records to be later studied and analyzed; the dispensaries and the surgical wards are teeming with suggestive opportunities. No extraordinary vision nor the inspirations of genius are required or demanded—all that is necessary is enthusiasm and the impetus toward and the capacity for work.

Serology is bristling with queries to be answered. Methods are proposed whose value can only be determined by analytic and critical trial; the value and significance of complement-fixation tests upon the cord blood is still a matter of dispute; the relative value and specificity of various antigens and technical modifications is open to study—and so on *ad infinitum*.

Even the records of the humble urinalysis may be scrutinized with profit, compared and correlated, perhaps, with the blood chemistry. Better results and better methods of reporting them are always within the realm of possibility. The enthusiasm of even one worker may serve as the means of awakening that of others. It is not sufficient to keep laboratory records—they should be studied and without fail will furnish plenty of food for thought and queries for elucidation. Even if nothing but the crystallization and clarification of one's own knowledge is accomplished, that in itself is something and often leads to further problems and activities.

It should be demanded and expected of every hospital laboratory and of its director that at least one paper embodying some investigative activities should come from it each year—nor should less than this be demanded of the clinical divisions.

When one sees a hospital, perhaps of some size, as one may—with an imposing list of admissions; with laboratories well equipped as to apparatus and personnel, and observes that the hospital report carries merely a tabulated list of the number and kind of laboratory examinations made and reports rendered, and fails to find in the index of current literature any mention of reported observations or investigations begun—there are only two possible explanations which come to mind: Either the knowledge and information gained from its work is closely and selfishly hoarded within the institution; or else its work and its personnel are fast sinking into a state of lethargy and innocuous desuetude undisturbed by any quiver of that acquisitive spirit of inquisitive scientific curiosity which leads to the endeavor, at least, to extend one's knowledge.

—*R. A. K.*

Infections Due to the Fusiform Bacilli and Spirochetes of Vincent

THE acute inflammatory condition first described by Vincent associated with the presence of fusiform bacilli and spirochetes in the lesions has, until rather recently, been regarded as a relatively infrequent and comparatively trivial infection.

Its occurrence, however, during the World War in extensive and even epidemic form as an ulcerative gingivitis in large bodies of troops has awakened and renewed interest in this condition and its causative organisms.

Prior to the experiences met with during the war this infection was generally associated mentally, particularly with infections of the tonsillar and pharyngeal areas; that it could be responsible for lesions limited to the gums was, perhaps, not generally appreciated.

The condition has always been of some interest bacteriologically because of the apparent indissoluble symbiotic relationship existing between the bacillus fusiformis and the spirochetes with which it was always found associated.

It was, apparently, not possible to grow these organisms separately in pure cultures in spite of repeated attempts by various workers.

This failure and this apparent symbiosis are believed by Tunnicliff[1] to be due to the fact that the organisms are really one and the same, the spirochetes being but a stage in the life cycle of the B. fusiformis. While this conclusion has been disputed by other workers and Tunnicliff's observations have not yet been confirmed or accepted by bacteriologists at large, the evidence presented which is based upon the study of pure cultures, cannot be lightly cast aside.

Partly because of isolated observations from which somewhat unwarranted conclusions have been drawn; because of the fact that syphilitics, are, apparently, somewhat susceptible to this infection; and because of the further fact that arsphenamine and its derivatives are extremely effectual in the treatment of the disease, being practically a specific, there has been—and especially among the dental profession—some tendency to consider the condition as related to or associated with syphilis in some way.

Thompson,[2] for example, is quoted as authority for the statement that a positive complement-fixation reaction occurs in Vincent's angina in the absence of syphilis; and this impression, which it is somewhat difficult to trace to its source, has gained some circulation. These findings, however, have not been corroborated by observers working with modern and perfected methods, and even failed of substantiation as long ago as 1918 by Taylor and McKinstry[3] whose studies were specifically directed toward this point.

Soberheim[4] has been often referred to with reference to the supposed relation between syphilis and Vincent's angina. His evidence, however, consists of three cases, two of which were very evidently concurrent infections, and in the third syphilis could not be ruled out.

Vincent's angina does occur with some frequency in syphilitics and especially in those whose dental hygienic conditions and individual reactions to specific therapy lead to the occurrence of stomatitis; whether or not it occurs more frequently in the syphilitic than in the nonsyphilitic, statistics are not available to indicate and the question is of relatively little significance other

than as indicating the favorable preparation for secondary invasions by the inflammatory reactions accompanying or initiating stomatitis due to excessive drug reactions.

By some observers the organisms of Vincent's angina are looked upon as essentially secondary invaders; opposed to this view, however, are the facts that: the organisms are found only infrequently in the clean and healthy mouth; they are common in infectious conditions of the teeth, gums, and tonsils; the disease may occur in epidemic form and it responds rapidly to neoarsphenamine with a simultaneous disappearance of both the lesions and the organisms.

There is, of course, as little reason for assuming a relation between the spirochetes of Vincent and the Spirocheta pallidum because both are destroyed by arsphenamine as for assuming a relation between the typhoid bacillus and the Staphylococcus because both are killed by bichloride of mercury solutions.

If gingivitis due to the organisms of Vincent occurs with relative frequency in syphilitics it furnishes simply another potent argument for the necessity for strict and thorough dental hygiene in syphilis.

Fortunately, arsphenamine and its derivatives furnish a practically specific method of treatment as ample evidence proves. Barenberg and Bloomberg[5] recently report brilliant results with sulpharsphenamine either locally or intramuscularly. While treatment has been given intravenously as well as intramuscularly, equally good results have been obtained by local applications of the drugs in suspension in glycerin.

It seems possible that the organisms responsible for this infection may be even more widespread than has heretofore been thought. Brams and Pilot[6] in a study of erosive balanitis, which condition, as is well known, is not uncommonly associated with, and may be due to the presence of fusiform bacilli and spirochetes, were led to make examinations by smear and culture of the preputial secretion of one hundred normal men with the demonstration of these organisms in 51 per cent. Similar examinations in women gave an incidence of 58 per cent of positive findings.

The frequent presence of these organisms in the teeth and tonsillar crypts has been noted by Davis and Pilot[7] and many others.

In view of these facts and of the extensively destructive lesions which these organisms are capable of producing, it is fortunate that specific as well as rapidly effectual methods of treatment are at hand.

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[1]Tunnicliff, R.: Life Cycle of B. fusiformis, Jour. Infect. Dis., August 1923, ii, 147.
[2]Thompson, St. Clair: Dis. of the Nose and Throat, Cassell, London, 1916, Ed. 2, p. 415.
[3]Taylor, F. G. and McKinstry, W. H.: A Serologic Investigation of Vincent's Angina, Jour. Laryngol., Rhinol. and Otol., London, 1918, xxiii, 289.
[4]Soberheim: Arch. f. Laryngol. u. Rhinol., 1909, xxi, 5.
[5]Barenberg, L. H. and Bloomberg, M. W.: Use of Sulpharsphenamine in Vincent's Angina and Stomatitis in Children, Jour. Amer. Med. Assn., July 5, 1924, lxxxiii, 25.
[6]Brams, J. and Pilot, I.: A Study of Erosive Balanitis etc., Arch. Dermat. and Syph., April, 1923, vii, 1.
[7]Davis, D. J. and Pilot, I.: Studies of B. Fusiformis and Vincent's Spirochetes, Jour. Amer. Med. Assn., September 16, 1922, lxxix, 944.

—*R. A. K.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

Medical Quotations[1]

HAVE you not often planned on card indexing remarks, phrases or paragraphs which you have come across in your general reading, bearing upon some subject of particular interest, be it religion, poetry, philosophy or science? This Dr. Lindsey has done in collecting random remarks on medical subjects by various English authors. These include Sir John Mandeville, Sir Thomas More, Francis Bacon, Robert Burton, Sir Thomas Browne, Samuel Pepys, Jonathan Swift, George Berkeley and Laurence Sterne.

Taken at random many have little significance. The quotations of Samuel Peyps, however, which comprise about two-thirds of the volume, are interesting and while they again are unrelated excerpts, they make thoroughly delightful reading.

International Clinics,[2] *June, 1924*

THE second volume of this series of International Clinics contains interesting articles on diverse subjects. The reviewer mentions particularly, one by Dorland on the influence of x-rays on bacteria, plants, protozoa, organic substances, butterflies, beetles and other insects, intestinal worms, marine forms of life, birds, reptiles and mammals. The experimental work done on normal tissues and on pathologic tissues is reviewed critically. The author emphasizes the dangers to the fetus in radiation of the pregnant mother either for diagnostic or for therapeutic purposes. A comprehensive bibliography is appended.

Balfour and Flynn summarize recent advances in surgery. To a great extent a compilation of recent work at the Mayo Clinic, its scope, is by no means thus delimited. While naturally of interest to the surgeon, the discussion will be of particular value to those interested in others of the medical specialties, in that all unnecessary detail has been omitted, and one may in a few short pages become reasonably well acquainted in a general way with recent surgical advance.

A contribution of distinct promise is that of Graham and Cole referred to by Balfour and Flynn on the study of gall-bladder disease by the intravenous injection of substances opaque to the x-ray and which are excreted through the bile.

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[1]Medical Quotations. From English Prose. John H. Lindsey, M.D. With illustrations. Cloth. Pp. 298. Price $2.50. Richard G. Badger, Boston, Mass. 1924.

[2]International Clinics, vol. ii. Series 34, June, 1924. A quarterly illustrated clinical lectures and especially prepared original articles. By leading members of the medical profession throughout the world. Pp. 306. Cloth. Price $2.50. J. B. Lippincott Company, Philadelphia, 1924.

International Clinics, September, 1924[3]

THIS volume conforms with its name in that we find herein several interesting contributions from European authors. An essay by Sherwood-Dunn on the evolution of the treatment of syphilis is of interest in that it enables us to contrast American methods with the continental routine. The conclusions are naturally quite similar but there appear some noteworthy variations in the standard course of treatment.

Another European contribution on the treatment of the various types of allergic disease serves to remind and to convince us that the real advances of the last few years in the treatment of asthma, hay fever and allied conditions, have been made especially by American investigators.

Hipwell and Gilchrist give a word of hope concerning insulin, in that they report a series of cases in which diminution of insulin dosage in the course of the treatment of diabetes has suggested very strongly the existence of truly curative properties.

Zingher summarizes recent work on scarlet fever, describing particularly the Dick test and the process of immunization. His description is accompanied by color plates.

Calorimetry in Medicine[4]

THIS is not a handbook for clinicians. The author has devoted no space to the technic of determination of the basal metabolic rate and has incorporated no tables for computation. The work is essentially a monograph on metabolism in health and disease rather than as the name would suggest, a treatise on the procedure of measuring heat production. The author emphasizes that the diagnostic use of basal metabolic rate determinations in dysthyroidism, while of unquestioned value, is probably of less importance than is the information to be gained from a study of heat production and heat regulation in various other pathologic states. He emphasizes that the study of total energy transformations in disease should no longer be confined to the postabsorptive state but that the effect of the ingestion of food and the cost of muscular work should receive further investigation.

The book is essentially a résumé and discussion of the valuable work of all investigators, including that of the author, on the basal metabolism in diseases such as pregnancy, nutritive disturbances, fever, diseases of the circulatory system, etc. The effect of drugs and particularly of the various foods on the basal metabolism is discussed.

The work should serve as a valuable reference guide in the clinical interpretation of the results of metabolism studies.

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[3]International Clinics. Vol. iii. September, 1924.

[4]Calorimetry in Medicine. By William S. McCann. Associate Physician, Johns Hopkins Hospital. Cloth. Pp. 98. Price $2.25. Williams & Wilkins Co., Baltimore, 1924.

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The Next Annual Meeting Will Be Held in Philadelphia May 21-23, 1925 Prepare for the Coming Convention

A letter and questionnaire have been sent out to all our members apprising them of our next annual meeting which is to be held May 21, 22 and 23, 1925, in Philadelphia, the home city of our President, Dr. Kolmer. Under his inspiration the gathering next year bids fair to excel our previous successful conventions in scientific contributions to our specialty and advancement of our cause. A new and useful feature will be the commercial exhibit of instruments, apparatus and reagents for the laboratory worker. Members should make plans now for attending our next meeting. Dr. Burdick will be pleased to make hotel reservations not only for the Philadelphia meeting of the A. S. C. P. but he is also in a position to extend the service to include reservations in Atlantic City for the A. M. A. convention which is held the week following ours. Those who have papers to present, and we hope there will be many, will communicate at once with the secretary, Dr. Ward Burdick, 652 Metropolitan Bldg., Denver, Colorado.